ECOPHYSIOLOGY OF A WILD NOCTURNAL PRIMATE, THE JAVAN SLOW LORIS (NYCTICEBUS JAVANICUS)

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Cover Illustration of a Javan slow loris by KD Reinhardt

The goal of this dissertation thesis was to bridge the gaps in knowledge and understanding of ecophysiology of a Critically Endangered primate, which can then be applied to conservation management and planning. I studied a population of wild Javan slow lorises (*N. javanicus)* ranging in a submontane agroforestry habitat in Cipaganti, West Java, Indonesia. I used a cumulative dataset configured of data collected by myself and various other trained members of the Little Fireface Project (LFP) research team between March 2015 and August 2018. Using external bio-logging equipment, I investigated topics of behavioural flexibility and physiological responses to the environment. In Chapter 3, I used skin temperature (T_{sk}) loggers for measuring the thermoregulation index of slow lorises. Slow lorises displayed sporadic use of daily torpor, with individual variation in torpor depth and minimum T_{sk} flexibility. In Chapter 4, I monitored and quantified sleeping site selection and its potential role as a thermal refuge for slow lorises as a selective pressure. Bamboo stands buffered ambient temperatures (T_a), providing stable microclimate temperatures for sleeping sites. In Chapter 5, I used accelerometers to examine slow loris sleep patterns and their intrinsic homeostatic sleep need. Sleep-wake patterns of slow lorises anticipated changes in light environment, where they began and ended activity patterns, as well as disruption of naps by changes in T_a. In Chapter 6, I monitored slow loris behaviour to examine how individuals acquire food energy in an anthropogenic habitat. I further monitored the phenology of their food sources to examine how their behavioural ecology role fits into the ecosystem. Slow lorises consumed floral nectars from an introduced leguminous plant *Calliandra calothrysus*, and contributed to the plant species' pollinator network in the Cipaganti area. The synthesis of data presented in this thesis suggest that the use of external bio-loggers is suitable (and least invasive) as a proxy method to measure physiology in wild primates. As an important food source that plays a key role in the regular behaviours and physiological responses of Javan slow lorises, *C. calothrysus* should be

considered for conservation re-wilding methods, including: reintroduction of slow lorises to new habitats with presence of *C. calothrysus*; as well as introduction of *C. calothrysus* as an ecological substitution to already established Javan slow lorises home ranges. The physiological responses of *N. javanicus*, particularly to climate and resources, should be considered when choosing translocation and reintroduction sites for rescued slow lorises. The data presented in this thesis overall suggests that *N. javanicus* is highly adaptable to environmental changes, with a broad scale of thermal flexibility to endure colder temperatures, and the ability to adapt to new food resources in maintaining regular energy intake, while possessing circadian rhythms and activity patterns that are mediated by an intrinsic biological clock, driven by the environment. In regards to evolutionary biology, these data support the hypothesis that heterothermy in primates is an ancestral trait from mainland Africa, before the divergence of lorisids. These data also suggest the sleeping patterns of slow lorises represent phylogenetic inertia for the evolution of human sleep. In conclusion, I suggest that some of our basic notions about physiological responses obtained in the lab may vary in the wild, but can also be applied to rewilding conservation for this critically endangered primate.

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Top: A photograph of the field team in Cipaganti. Bottom: A photograph of LFP staff, wearing t-shirts provided by funder Mohamed bin al Zayed Species Conservation Fund (left to right, D Rustandi, KD Reinhardt, A Nunur, MY Nazmi).

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ABBREVIATIONS

Asl—above sea level
BMR—basal metabolic rate
CR—Critically Endangered
CBD—Convention on Biological Diversity
COP—Conference of Parties
DD—Data Deficient
CR—Critically Endangered
df—number of degrees of freedom
DBH—diameter at breast height
EEG—electrocephalogram
EN—Endangered
ENG—electronystagmography
GPS—Geographical Position System
ha—hectare
IUCN—International Union for the Conservation of Nature
LC—Least Concern
LFP—Little Fireface Project
MR—metabolic rate
mya—million years ago
NGO—Non-governmental organization
NREM—non-rapid eye movement
REM—rapid eye movement
RSFs—Resource Selection Functions
SD—Standard deviation
SE—Standard Error
SEM—standard error of the mean
SWS—slow wave sleep
T_a—ambient temperature
T_b—body temperature
T_i—internal temperature
T_o—outer temperature
T_{sk}—skin temperature
TST – total sleep time (synonymous to 'sleep duration')
VU—Vulnerable

Table 1.1. Vocabulary and definitions of key terms.

Vocabulary	Definition
Cathemeral	When sleep and behavioural activities are equally distributed between light and dark phases over a 24h period; often described as irregular distribution of activity patterns, categorising an animal as neither nocturnal nor diurnal
Crepuscular	When behavioural activities are primarily performed during hours of twilight
Diurnal	when an animal displays the majority of their behaviours and activity patterns during the day
Ectotherm	An animal that cannot regulate body temperature, and so fluctuates considerably with their environment (relying on external heat sources)
Endotherm	animals that maintain their core body temperature and homeostasis by means of thermoregulatory heat production
Energetics	The balance and redistribution of energy intake/output by an organism
Haplorhini	A suborder of the Order Primates, containing anthropoids (simians) and tarsiers, divergent from the Strepsirrhines; "dry-nosed" primates
Heterothermy	The physiological trait of self-regulating body temperature and adjusting to the immediate environment
Hibernation	a physiological state of hypometabolism, where an animal remains in a state of torpor (see definition below) for multiple days/months, displaying hypometabolism at a fraction of their basal metabolic rate; most often observed to cope with harsh winter seasons.
Homeostasis	maintenance of life-supporting physiological processes of the body in equilibrium
Homeothermy	An animal that maintains a relatively stable body temperature, maintaining thermal homeostasis.
Hyperthermia	when T_b levels exceed an animal's upper limitation, due to the body absorbing more heat than it can dissipate
Hypothermia	when T_b surpasses an animal's lower limitations, due to the body dissipating more heat than it can absorb
Strepsirrhini	A suborder of the Order Primates, including lorises, lemurs and galagos; "wet-nosed" primates
Lorisidae	A family of Strepsirrhini including all African and Asian lorises (lorises, pottos, angwantibos)
Lorisiformes	An infra-order of nocturnal primates, including lorises, galagos, pottos and angwantibos
Lorisinae	A subfamily of Strepsirrhini including slow (*Nycticebus* spp.) and slender (*Loris* spp.) lorises
Monophasic	When sleep is performed in a single bout, during a 24h period
Nocturnal	When an animal displays the majority of their behaviours and activity patterns at night
Normothermia	the condition where an individual is within their normal T_b range for
Phenology	life cycle of the flowering and fruiting of an individual plant or population
Phylogenetic inertia	A phylogenetic constraint, limiting future evolutionary pathways due to previous adaptations
Physiology	the study of life-supporting bodily functions, required for an organism to live
Plesiomorphic	When taxon share an ancestral trait with earlier clades of an order
Polyphasic	When sleep is divided into 2+ phases during a 24h period
Prosimii	The suborder of Primates, that includes Strepsirrhini and Tarsiiformes; means 'wet-nosed primate'
Tarsiiformes	An infra-order of nocturnal primates belonging to both the suborder Haplorhini as well as Prosimii (in earlier classifications)
Torpor	A physiological state of lethargy, where an animal notably reduces their metabolic rate, body temperature, heart rate and oxygen consumption. Can range from daily heterothermy (>24 hr duration) to hibernation (<month).

CHAPTER 1. GENERAL INTRODUCTION

Beginning over two centuries ago, human activities (including deforestation, natural resource exploitation and landscape changes) have created such an imprint on natural processes of the environment, that the current geological epoch has been termed the 'Anthropocene' (Crutzen & Stephen, 2003; Crutzen & Stoemer, 2000). Naturally, an animal must balance their energetic requirements against the fluctuations of their immediate environment, and periodic changes in an ecosystem (Schmidt-Nielsen, 1997). However, the Anthropocene requires wildlife to cope with acute anthropogenic changes, where land has been modified for agricultural use or deforested for logging, and fossil fuel exploitation has accelerated climate change across ecosystems on a global scale. The degree to which an animal can adapt to these additional changes will depend on a combination of behavioural flexibility, physiological mechanisms and limitations (particularly thermal flexibility) and their ecological niche (Brett *et al.*, 2014). In this thesis I bring these concepts together by examining a Critically Endangered (CR) primate facing severe anthropogenic changes, the Javan slow loris (*Nycticebus javanicus*). This introduction will cover the key topics of this thesis within the disciplines of anthropology and biology, including behavioural flexibility, thermoregulation and sleep, from perspectives in evolutionary biology and conservation. Studying wild animals in the Anthropocene (where fully intact forests are few and fleeting) may help us to conserve their existence in the wild, by knowing their physiological limitations and adaptability.

1.1. THERMAL STRATEGY

Endotherms are animals that maintain their core body temperature (T_b) and homeostasis by means of thermoregulatory heat production, and includes all species of mammal (see Table 1.1). Maintaining T_b in itself is energetically costly, but is required for proper cell function, reproduction and thus, survival (Geiser, 2013; Martin, 2001). The ability to produce heat allows an animal to inhabit various environment types and thermal niches across the globe, while maintaining normothermia (Blanco *et al.*, 2018; Lovegrove, 2012; Lovegrove *et al.*, 2014; McNab, 1978; Moyes & Schulte, 2008). Normothermia is a narrow range for animals and is on average around 37 °C for mammals (Heldmaier *et al.*, 2004). Nevertheless, climatic and seasonal variations can create stress on normal behaviours, and animals must adapt to avoid deviating from normothermic T_b levels, as this can lead to pathophysiological states, resulting in physiological damage or death of an individual (Terrien *et al.*, 2011).

Temperature changes can have drastic effects on physiological processes, and taxa vary greatly in their tolerance of temperature ranges, as well as across their life cycle. The thermoneutral zone (TNZ) is the range of environment temperatures in which an animal can sustain behavioural efficiency with minimal energetic costs or physiological stress (Romanovsky *et al.*, 2002). When an endothermic species experiences environmental temperatures deviating from the TNZ, they can control heat loss and production using a combination of methods such as physiological responses, behavioural thermoregulation or activity adjustments (McNab, 1982). The combination of behavioural and physiological response types is referred to as an animal's thermal strategy, ensuring their T_b remains within their physical limits (Moyes & Schulte, 2008).

1.1.1. HETEROTHERMY: TORPOR AND HIBERNATION

Endothermy is understood to have evolved independently at least twice in the Animal Kingdom: once in birds and once in mammals, each originating from distinct reptilian lineages, with bird endothermy having evolved much later in time (Geiser, *et al.*, 2002, 2004, 2013; Lyman *et al.*, 1982; Moyes & Schulte, 2008). Endothermic animals regularly exhibit low levels of what is called hypometabolism during periods of inactivity, where an individual lowers their normal metabolic rate (MR) by an average of 20%, and most often in conjunction with lowering T_b by ~2°C (Heldmaier *et al.*, 2004). While endotherms display this slight flexibility of internal temperatures, metabolic control of T_b beyond the range of normothermia is limited to heterothermic species. Heterothermic animals are endotherms with the capacity to adjust their body temperature (T_b) and MR to their environment, in a state of hypometabolism. Heterothermy is an ancestral mammalian trait to cope with environmental stressors (Lyman, 1972; Rial *et al.*, 2010). The evolutionary drivers of this physiological trait are still debated, as to whether it is retained from ectotherms (vertebrate species whose T_b varies considerably, conforming with environmental temperatures), or if it is convergent with heterothermic traits we see in birds (Rial *et al.*, 2010). This trait is also hypothesised to have derived in the Cretaceous period during times of thermal radiation (Lovegrove, 2012; Lovegrove *et al.*, 2014) further supported by Blanco *et al.* (2018), who suggest heterothermy derives from tropical environments first and foremost. This hypothesis was derived from research conducted on various species of primate inhabiting tropical environments that regularly use torpor and hibernation (Blanco *et al.*, 2018).

In environments with extreme climates or seasonal stressors (i.e. when resources are scarce), heterothermic animals can conserve energy by using a prolonged period of hypometabolism, such as torpor—a state of lethargy where an organism notably lowers its

metabolic rate, core T_b, heart rate and oxygen (Geiser, 2004; Ruf & Geiser, 2015). Animals that use this mechanism vary from levels of daily torpor (<24 h; often referred to as daily heterothermy) to multiday torpor (2-3 days) or hibernation (multiple months; often seasonal). In regard to endothermic animals, torpor and hibernation are the most effective method for conserving energy in demanding environments (Geiser, 2013).

Daily heterotherms will enter a torpid state for a few hours during part of the day and at a lesser degree—most frequently defined as animals lowering their T_b below ~30 °C, but not lower than 15°C (Geiser, 2004). Hibernators on the other hand, will remain torpid for multiple days or an entire season, and are characteristic of lowering their T_b well below 15°C, closer to 5°C (obligate or 'true' hibernation; Lyman *et al.*, 1982). During the process of hibernation, an individual reduces their metabolic rate to a fraction of their basal metabolic rate. This process is usually joined with lowering their T_b well below normothermia, although some species only lower their T_b minimally (e.g. *Ursus* spp.: Geiser, 2004; Lyman *et al.*, 1982) or even above 30°C during a hibernation bout (e.g. *Cheirogaleus medius*; Dausmann *et al.*, 2005). Hibernators rely heavily on metabolic inhibitions in addition to T_b adjustments to reduce their metabolic rate, whereas daily heterotherms can rely more on T_b to lower their metabolic rate (Geiser, 2004).

1.1.2. BEHAVIOURAL THERMOREGULATION

When an endothermic animal is exposed to extreme heat (e.g. high ambient temperatures, wild fires or exercise-induced) for an extended period of time, it experiences abnormally high T_b levels that can lead to hyperthermia (Adolph, 1947). Hyperthermia can cause various deleterious effects, as prolonged heat stress can damage the central nervous system of the brain (Marino, 2004), and accelerate metabolism and water loss (Rowell,

1990). To avoid hyperthermia, mammals can dissipate excess body heat through the process of thermolysis, bringing T_b levels back down towards normothermia (Nagashima, 2006). Thermolysis mechanisms include sweating (Šumbera *et al.*, 2007), panting (Goldberg *et al.*, 1981; Robertshaw, 2006) and vasodilation (Wood, 1991). Using behavioural thermoregulation can aid the process of thermolysis to dissipate heat with minimal energetic use, as part of the thermal strategy. Behavioural thermoregulation is when an animal adjusts their expressed proportion or behaviours over time, or adjusts their body posture or location to maintain normothermic T_b (Carey *et al.*, 2003; Lyman *et al.*, 1982). To reduce movement or activity over an extended proportion of time during an animal's activity period can decrease their average energy costs and allow an animal to tolerate higher temperatures than usual (Moyes & Shulte, 2008; Ostner, 2002). Main observations have included: elongated postures to increase surface area for promoting evaporation of heat (Donati *et al.*, 2011; Whittow, 2013); enforced resting and/or decreased energy intake to decrease heat production (Ishii *et al.*, 1996); maximising conductive heat loss to the air or a cooler substrate (e.g. contact with rock or ground) (Flouris, 2011; Gale *et al.*, 1970; Hafez, 1964); muscular movements of highly vascularised surfaces of skin (Phillips & Heath, 2001) or moving to cooler places that provide thermal refuge (e.g. burrow or tree hole) (Almeida *et al.*, 2006).

Contrastingly, should an animal experience abnormally low T_b (below normothermia) for a prolonged period of time, they risk experiencing hypothermia. One short-term solution to maintaining normothermia in cold environments is through the engagement of shivering thermogenesis (Carey *et al.*, 2003; Lyman *et al.*, 1982). Shivering thermogenesis is a physical reaction, where the muscles contract in order to produce body heat in maintaining T_b (Carey *et al.*, 2003; Lyman *et al.*, 1982; Moyes & Schulte, 2008). Shivering is an

energetically costly process, although similarly to thermolysis, its energetic costs can be minimised through aid of behavioural and physiological mechanisms (Heldmaeir, 1989). Behavioural thermoregulation can be used to manage accelerated heat loss (Heldmaeir, 1989). Locomotor adjustments can also generate heat (Whittow, 2013), with increased locomotion generating immediate muscle heat (Zerba & Walsberg, 1992). Diurnal placentals and marsupials will use sun-basking, where the individual sits in direct sunlight to absorb heat from radiation emitted from the sun, warming the individual's surface (Hillman *et al.*, 1998; Moyes & Schulte, 2008; Whittow, 2013). The level of thermal energy gained from this method will vary between species and individuals, depending on the surface area of skin or fur structure, density or even colour (Kleiber, 1947; Olfaz *et al.*, 2011; Wacker *et al.*, 2016).

Hair, fur and feathers aid in preventing heat loss in low temperatures, by decreasing the flow of air across the surface area of the skin that would create convection heat loss. Thicker hair structure and density of hair (coats) will increase the insulation capacity of hair against heat loss (Wacker *et al.*, 2016). By changing the direction of hair against the direction of air flow will also aid in retaining heat by decreasing the amount of air that can reach the skin's surface (Chappel *et al.*, 1989; Dawson *et al.*, 2004). However, ambient temperatures can often diverge beyond the threshold for which these anatomical features can help (Terrien *et al.*, 2011). Similar to an individual using behavioural thermoregulation to dissipate heat, an animal can use postural adjustments to conserve body heat, by alternatively decreasing the body's surface area that is exposed to the external environment (Oritsland, 1970; Schmidt, 2011). This type of postural thermoregulation most often involves resting with the head curled under the abdomen, to keep the heart warmer than the body while still allowing blood flow to the abdomen (Lyman *et al.*, 1982).

Furthermore, animals may decrease their surface area to volume ratio via postural adjustments with conspecifics, such as huddling, or social thermoregulation (Fedyk, 1971; Gilbert *et al.*, 2010; Heldmaeir, 1975; Ijzerman *et al.*, 2015; Williams *et al.*, 2013).

1.2. SLEEP

Sleep is a fundamental requirement for many animals in maintaining cognitive and physiological functions (Kushida, 2004; McNamara *et al.*, 2010; Rechtschaffen, 1998). The quality of sleep in which an animal can experience is influenced by environmental temperatures, and is significantly reduced when an animal experiences temperature diverging from their TNZ (Heller, 2005; Heller *et al.*, 1983; Rial *et al.*, 2010; Siegel, 2005). This effect is exemplified in smaller mammals, as they experience more heat loss due to a larger surface area, and tend to have higher metabolic rates (Elgar, 1989). Heat loss during sleep can be avoided by shorter and frequent sleeping bouts, behavioural thermoregulation, or sleeping in locations that retain heat, such as a thermal refuge (nests or tree holes) (Eppley *et al.*, 2017; Goldingay, 2015; Lutermann *et al.*, 2010; Schmid, 1998). Therefore, the thermoregulatory capacities of a species are hypothesised to impact behavioural thermoregulation and microhabitat use, such as sleeping site selection (Rezende & Bacigalupe, 2015).

1.2.1. SLEEPING SITES

The majority of birds and mammals spend nearly half of their time at sleeping sites, and so, a variety of evolutionary pressures are considered to be key drivers in the selection of these sites (Allison & Cicchetti, 1976; Anderson, 1998; Capellini *et al.*, 2008; Lesku *et al.*, 2006). Sleep patterns expressed in mammals are correlated with ecological factors, such as safety, shelter, body mass and diet (Lima *et al.*, 2005; Siegel, 2005). Thus, sleep is governed by an animal's ecological niche and sleeping site characteristics (Anderson,

1998; Lima *et al.*, 2005). The selection of a sleeping site must be considered from an evolutionary perspective, where various factors may play important roles in shaping selective patterns. In primates, sleeping sites have most commonly reflected structure that aids in avoiding detection from predators (Heymann, 1995; Reichard, 1998), proximity to food resources (Anderson, 1984; Chapman, 1989; Chapman *et al.*, 1989; Cowlishaw, 1997), maintaining territory (Tilson & Tenaza, 1982), and shelter from weather variables (Aquino & Encarnación, 1986; Schmid, 1989).

A sleeping animal is at a higher risk of predation, due to a reduced level of awareness and responsiveness, and this can lead to immediate death (Allison & Cicchetti, 1976; Lima *et al.*, 2005; McNamara *et al.*, 2010). This makes predation one of the major pressures influencing sleeping site location in mammals (Anderson, 1998; Lumsden, 1951). Site characteristics associated with predator avoidance by primates include decreased detectability, non-accessibility and escape routes (Anderson, 1998; Reichard, 1998). Researchers suggest that terrestrial primates experience equal levels of predation risk during sleep compared to arboreal primates (Isbell, 1994), which drives the selection of above-ground sleeping sites (in trees, nests, tree holes, cliffs) in all primates (Anderson, 1984), except gorillas (Cheney & Wrangham, 1987) and lemurs that burrow underground (Kappeler, 1998). Gregarious primate groups will frequently choose sleeping trees with terminal branches that are either located high above tree trunks or hanging over cliffs, rivers and streams, proving difficult for predators to access as well as alternative routes, that can aid in avoiding approaching predators (Anderson, 1998; Goodall, 1962; Yoneda, 1984).

As sleeping animals are also susceptible to changing environment conditions, the structure of a sleeping site is important for providing shelter from daily climatic variables, including wind-chill, rainfall, or snow (Aquino & Encarnación, 1986; Kappeler, 1998). Some areas display a pronounced microclimate, that is far more suitable for an organism or species' population (Bramer *et al.*, 2018). A microclimate is an area displaying climatic variations (on a finer scale) deviating from the overall atmosphere (macroclimate), ranging from a combination of climatic variables (e.g. temperature, humidity, cloud cover, rainfall, slope, wind chill/direction, solar radiation) created by topographic and vegetation differences (Bird *et al.*, 2002; Bramer *et al.*, 2018; Gates, 1980; Porter & Gates, 1969; Tracy, 1976). Therefore, choosing sleeping sites that buffer against external environmental variables is also crucial for the overall survival of an organism during sleep (Cowlishaw, 1994).

Most ecosystems have spatial variation, in which various parts of an environment can provide a distinct microclimate pocket during unfavourable climatic events (Bramer *et al.*, 2018). Microclimate area can range from the broader context of topographic variation, such as geographic slope or elevation levels, to the smaller scale of microhabitat structures or microrefugia, such as caves or orientation of vegetation (Bramer *et al.*, 2018). Mammalian taxa have been observed to use microclimates as thermal refuge across a diverse range of environment types and geographical scale, from pikas (*Ochotona* spp.) ranging at high altitude in the Trans-Himalayan mountains (>3,500 m asl; Namgail *et al.*, 2013) to primates ranging at sea level altitudes (e.g. mouse lemurs, *Microcebus spp*.) in tropical forests on the island of Madagascar (Kappeler, 1998; Lutermann *et al.*, 2010). Microhabitat structures can provide a unique microclimate and thus, thermal refuge, due to insulation that can maintain heat and/or buffer extreme climatic or environmental shifts, protecting an animal against sudden weather changes or low ambient temperatures

(Donati *et al.*, 2011; Entwistle *et al.*, 1997; Eppley *et al.*, 2017; Goldingay, 2011; Schmid, 1998).

1.2.2. SLEEP REGULATION

Animals have diverse photoreceptors and spectral sensitivities (Sliney, 2016). The detection of light by an organism's retinal receptors not only forms their vision, but profoundly drives behaviour and physiology, including circadian rhythms, sleep regulation and hormonal regulation (Fisk *et al.*, 2018). While influenced by light environment, these physiological responses are non-visual, representing an organism's entrainment to light (Peirson *et al.*, 2018). Virtually all organisms display some form of biological clock, where their physiology and behaviour display daily rhythms (Mercer, 1998). These rhythms allow an animal to interpret and predict the rhythmic changes in their environment (Jud *et al.*, 2005; Kavanau & Peters, 1976). As hypometabolism is often a seasonal response, this too can be anticipatory, demanding an organism to balance their temperature regulation and meet sleep needs with their circadian rhythm (Kavanu, 1997).

Animals can regulate their sleep through compensation for the loss of sleep following sleep deprivation (sleep homeostasis: Rechtschaffen, 1998; Tobler, 1985). Sleep rebound is a restorative function of sleep, where an animal reallocates time for sleep during their regular activity patterns if they experience a disturbance or prevention of sleep (Rattenborg *et al.*, 2004; Rechtschaffen, 1998; Rechtschaffen & Bergmann, 2002; Siegel, 2003; Tobler, 1985; Tononi & Cirelli, 2003). Sleep regulation must further be balanced against the fluctuations of an animal's environment, including temperature fluctuations, seasonality and light environment (Davimes *et al.*, 2018; Gravett *et al.*, 2017; Rattenborg *et al.*, 2008). Light environment is also related to seasonality, which allow animals to anticipate seasonal

rhythms in their environment, including changes in temperature and food resource availability (Mercer, 1998).

1.3. ECOPHYSIOLOGY

Animal physiology has long been researched in laboratory environments, where experiments can control for varying responses and adaptations of an animal. However, physiological responses and tendencies differ to those of the same species in the wild (Calisi & Bentley, 2009; Dausmann, 2005; Geiser, *et al.*, 2000, 2004; Herreid, 1963; Tomasi & Horton, 1992; Wilson *et al.*, 2015). Ecological physiology (hereafter referred to as 'ecophysiology') is the study of physiological functions and their purposes in both the maintenance and adaptability of an individual organism in response to its natural environment (Feder, 1987). Gradually over time, the aims in this field of research broadened to understanding not only the individual, but also the evolutionary pathways of various physiological traits and their drivers in nature.

Collecting direct behavioural observations on wild animals has proven challenging due to animal habituation, influence of observer presence on natural behaviours, or even limited detectability due to cryptic behaviour (Altmann, 1974; Brown *et al.*, 2013; Caine, 1990; Chimienti *et al.*, 2016; Crofoot *et al.*, 2010). Over the past few decades, the use of bio-logging technology has greatly expanded to cope with these challenges, increasing the possibilities for data collection in natural environments. Bio-logging has been used to record an animal's ranging behaviour and daily activity patterns (van Oort, 2004), energy expenditure (Cooke *et al.*, 2004a, 2004b), and even physiological parameters, such as body temperature (Dausmann, 2005; Hedd *et al.*, 1995), sleep (Rattenborg *et al.*, 2008; Rattenborg *et al.*, 2017; Serge, 2010) and heart rate (Ropert-Coudert *et al.*, 2006, 2009).

Expanding research in the field has brought us to learn that some species use torpor regularly in the wild, or only in response to extreme environmental changes (Geiser 2013; Nowack *et al.*, 2013). These environmental responses would explain why researchers are discovering more species to use torpor, as captive settings will have provided constant temperatures and food resources (Heldmaier & Ruf, 1992; Langer & Fietz, 2014). Body temperature (T_b) can aid in the interpretation of various physiological processes in animals, including hypometabolism (Bartholomew, 1962). To measure T_b, most researchers have used implanted temperature sensors to record an animal's core T_b in laboratory settings, with little long-term research conducted in natural settings (Heldmaier & Ruf, 1992; Langer & Fietz, 2014). Implantations prove complicated in wild settings including difficulty of initial capture and re-capture, sterilisation of operative procedures, post-operative monitoring of animals, and risk of altering home ranges or thermoregulatory processes (Audet & Thomas, 1991).

Another least-invasive method, accelerometers, have also been recognised as a powerful tool, frequently used to register behaviours and activity patterns in animals, including humans. This research has allowed comparison between various applications of accelerometer devices, and between species. While captive studies have advanced the validation of accelerometer use in primate research, wild animals are expected to display marked differences in behavioural and physiological responses in comparison to captive animals (Calisi & Bentley, 2009). Furthermore, researchers must consider the ethics of invasive research, particularly towards threatened species (categorised as such on the IUCN Red List), thus demanding alternative and improved methods to measure these

physiological parameters. Having covered these topics, I will now present information on conservation in the Anthropocene, moving on to how I connect them throughout the thesis.

1.4. CONSERVATION IN THE ANTHROPOCENE

Conservationists regularly seek to understand the roles and relationships of taxa within a functioning ecosystem, and how these roles interact in sustaining that ecosystem (Soulé, 1986). With ecosystem restoration currently recognised as a primary focus in global conservation planning, the Convention on Biological Diversity (CBD) has formulated attainable goals on a global scale to "rehabilitate and restore degraded ecosystems and promote the recovery of threatened species" (CBD Convention Text and Article 8(f), 1992). These goals were set out by the Conference of Parties (COP) comprised of non-governmental organizations and intragovernmental organizations around the world to implement biodiversity targets, climate change mitigation and sustainable development (Aronson & Alexander, 2013).

In 1998, rewilding practices were proposed as large-scale conservation approaches, receiving intense debates as to its impact and feasibility (Foreman, 2004; Soulé & Noss, 1998). Rewilding conservation involves the management of an ecosystem by introducing closely related species as a proximate for extinct species (Donlan *et al.*, 2005). The premise and motive of this methodology is to maintain or restore natural ecological processes, as opposed to managing extinction. The term rewilding has been used in conjunction with assisted migration, back-breeding, de-domestication, de-extinction, ecological restoration, naturalistic grazing, natural recolonization, reintroduction, taxon substitution, and translocation (Lorimer *et al.*, 2015). All of these methods are varying forms of rewilding conservation and land management of an ecosystem.

The introduction, reintroduction or translocation of vertebrate into an ecosystem has become common practice amongst conservationists, particularly with captive animals in zoos, or confiscated animals at rescue centres (Kleiman, 1989). Reintroductions are specifically referred to as "the release of animals held in captivity, both wild- and captive-born" (Kleiman, 1989). Where captive-breeding programs provide viable populations for reintroduction, a population can be released at a particular site, in hope of maintaining a stable population in the wild. Reintroductions can have a positive impact on threatened populations of endangered species, as has been demonstrated with the successful reintroduction and subsequent translocations of diverse species, including bison in North America and mainland Europe (i.e., American bison *Bison bison* and European bison *Bison bonasus*; Conway, 1980; Kleiman, 1989; Wemmer & Derrickson, 1987) as well as endangered tropical primates (i.e. Golden Lion Tamarin *Leontopithecus rosalia*; Kleiman *et al.*, 1986). However, overall attempts for mammalian reintroductions prove complicated—particularly, in finding suitable habitat for the more specialised and endangered species. Furthermore, animals may be misidentified, potentially being released into habitats outside of their native geographic range, not suitable for their physiological adaptations or ecological niche (Kleiman, 1989).

One of the primary results of the Anthropocene includes a mass extinction of native species, where animals must adapt to a loss of resources as well as an abundance of introduced species (ecological substitutes), often non-native (Caro *et al.*, 2012). For classification purposes in conservation biology and restoration ecology, a 'native species' is categorised as one that has auto colonised in a habitat, whereas a non-indigenous 'alien species' is one that humans have introduced, directly or passively (Hall, 2003; Low, 2002;

Warren, 2007). Humans may often introduce alien (non-native) species into a new area for economic gain, to increase agricultural profit, improve tourist attractions, or because they believe the non-native species will fill a role more efficiently than native species in a habitat (McNeely, 2011).

It has been found that introduced plant species (i.e. ecological restoration) will compete with native species for flower-visitor abundance (Brown *et al.*, 2002; Ghazoul, 2002; Larson *et al.*, 2006), sometimes having a negative effect on native species sustaining in a given ecosystem (Lopezaraiza–Mikel *et al.*, 2007; Moragues & Traveset, 2005). The survival of introduced species in competing with native species depends on the foraging behaviour of native pollinators, in their frequency of visits and quality of pollen transfer or deposits (Ghazoul, 2002). Based on their energetic needs, animals will adjust their foraging behaviours and food intake with their energy expenditure (McNab, 1980b). Should native pollinators shift their foraging preference to introduced species, this would influence fertilization of plants (Larson *et al.*, 2006). Finally, if these species of flora prove successful fruit sets in a non-native environment, it would suggest that this plant is a potential species for re-wilding, proving fit and sustainable in an introduced environment as an ecological substitute. Slow lorises are amongst the many endangered species faced with the complexities of rewilding, including frequent reintroduction of slow lorises from captivity to new wild habitat (often mistakenly outside of their native geographical range), as well as the need to adapt to ecological substitutes in their native home ranges (introduced for agricultural practices; see section 2.2.1).

Figure 1.1. Female juvenile Javan slow loris, named Tombol emerging from a bamboo stand after a harsh rain. Photo by: KD Reinhardt.

1.5.1. TAXONOMY AND EVOLUTION

The Order Primates is categorised into two suborders: Strepsirrhini and Haplorhini. An earlier classification frequently used for this Order were the division of Prosimii (Strepsirrhines and Tarsiiformes) and Anthropoidea (Platyrrhini and Catarrhini) (Martin, 1990). Earlier evolutionary phases of the suborders Strepsirrhini and Haplorhini are commonly investigated through an extinct group of primates, Adapoidea, from the earlier Paleogene. Phylogenetic analyses have suggested that Adapiformes represent stem strepsirrhines (Gebo, 2002; Herrera & Dávalos, 2016; Seiffert *et al.*, 2018), while morphological similarities to anthropoid taxa suggest that Adapiformes represent haplorhines (Gingerich *et al.*, 2010; Rasmussen, 1990, 1994; Bloch *et al.*, 1997; Franzen *et al.*, 2009).

The suborder Strepsirrhini is comprised of wet-nosed primates, phylogenetically considered the earliest of the living primates (basal primates), which can provide insight into primate origins and evolution. Strepsirrhini can be further divided into Lemuriformes (lemurs) and Lorisiformes (lorises, galagos, pottos and angwantibos). Within Lorisiformes, lorisids and galagids are considered to be sister clades, separating between the early Eocene and Miocene, dating back 54.9—23 mya (Andrews *et al.*, 2016; Herrera & Dávalos, 2016; López-Torres & Silcox, 2019; Munds *et al.*, 2018; Porter *et al.*, 1997; Pozzi *et al.*, 2014, 2015; Roos *et al.*, 2004; Seiffert *et al.*, 2018; Yoder, 1997; Yoder & Yang, 2004). While some researchers have hypothesised that lorisids originated in Asia (Martin, 2000, 2003; Pickford, 2012), fossil records and reconstruction of the galagid radiation suggest that lorisids are more likely to have originated in Africa (Masters *et al.*, 2005, 2007; Munds *et al.*, 2018; Pozzi *et al.*, 2015; Seiffert, 2012; Seiffert *et al.*, 2018). An African origin for lorisids is further supported by geological records and the forming of the *Gomphotherium* Land bridge, which connected mainland Africa and Eurasia through the Arabian plate (see Figure 1.2; Rögl, 1999; Sen, 2013). This formation united the Mediterranean Sea and the Indian Ocean during the closing of Paratethys in the late Miocene (Harzhauser *et al.*, 2007; Reuter *et al.*, 2009; Rögl, 1998, 1999). The oldest fossil record of lorisids found in Asia dates back to the middle of the Miocene between 14 and 13 mya (Nekaris & Rasmussen, 1998), which suggests that lorisines migrated to Asia during this geological event, through Afro-Arabia and the land bridge formation (Chaimanee *et al.*, 2008; López-Torres & Silcox, 2019; Mein & Ginsburg, 1997). It is further suggested that other African groups also used this land bridge to cross from Africa to Asia, including stem hominoids (Reichard *et al.*, 2016).

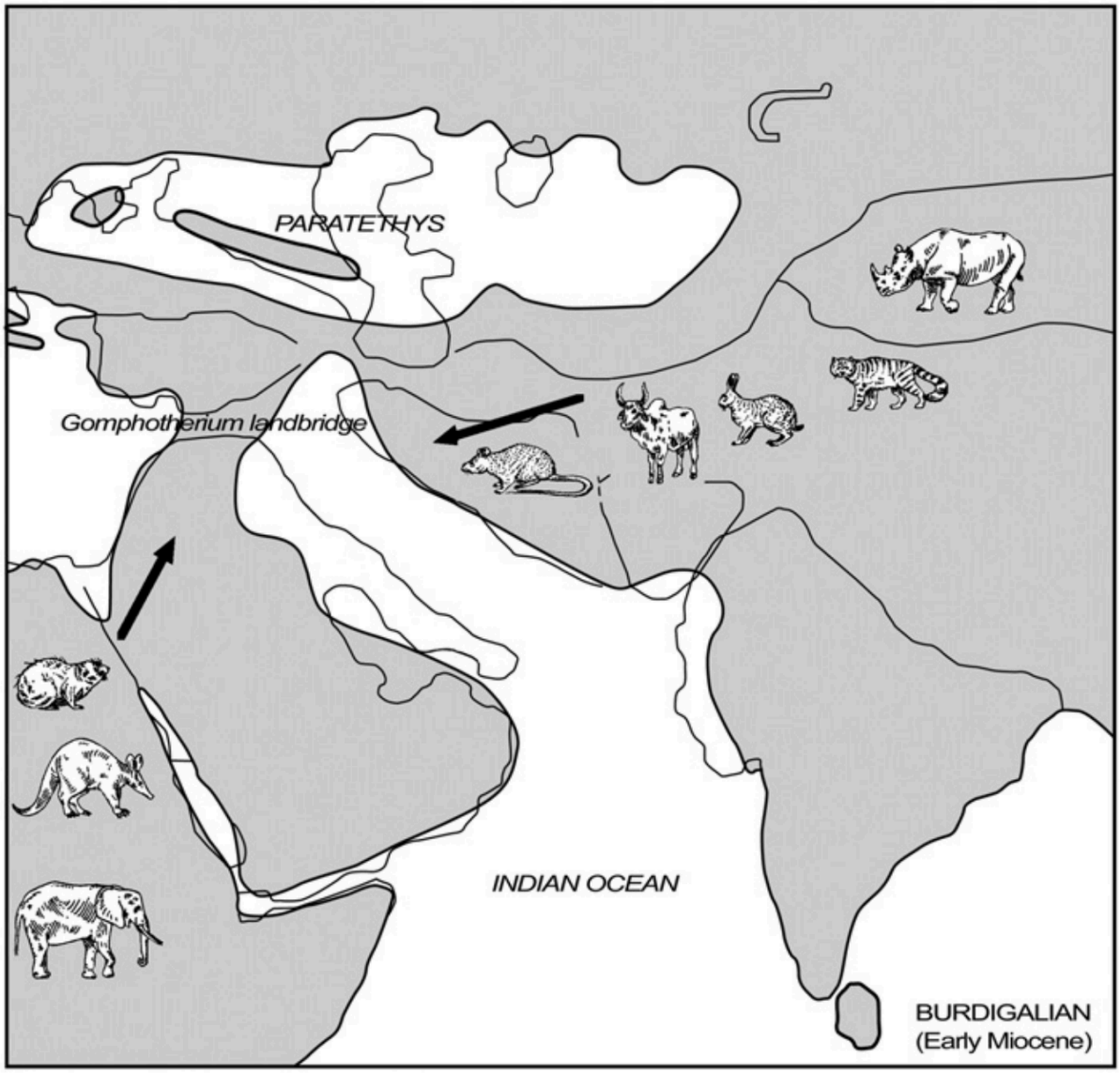


Figure 1.2. A map after Rögl, 1999, modified by Sen *et al.*, 2013 (added mammalian illustrations), representing the formation of the *Gomphotherium* land bridge during the early Miocene. Animals and arrows indicate the main mammalian groups involved in faunal exchanges between Africa to Eurasia. Mammals indicated with an arrow pointed towards Paratethys represent groups which would have crossed from mainland Africa towards Eurasia, using the land bridge. This group is suggested to have included lorisines.

The subfamily of Lorisinae (within Lorisiformes) is comprised of slow (*Nycticebus* spp.) and slender (*Loris* spp.) lorises, with molecular data suggesting a separation of the two between 21.14 and 4 mya (Perelman *et al.*, 2011; Seiffert *et al.*, 2018). Currently, there are nine recognised species of slow loris, all of which are listed as Vulnerable, Endangered or Critically Endangered on the IUCN Red List. All genera of *Nycticebus* range across South and Southeast Asia (Table 1.2), with some species being endemic to islands and all species inhabiting a diverse range of habitat types, including lowland tropical forest, montane forest, Acacia shrubland, peat swamp and anthropogenic landscapes (Barrett, 1981; Nekaris & Bearder, 2011; Nekaris *et al.*, 2014; Rode-Margono *et al.*, 2014). Despite ranging in tropical climate regions, known slow loris populations are distributed across a large altitudinal scale, from 0—2,000 m asl. This altitudinal range can display extreme seasonal variation between wet and dry periods, or daily temperature variation with an overall range of 7—50°C observed between habitats (Nekaris, 2014; Reinhardt *et al.*, 2016; Starr *et al.*, 2012; Streicher, 2004).

Table 1.2. A list of all nine currently recognised species of *Nycticebus*, listing their Latin name, common name, countries of geographic range and IUCN status.

Species	Common name	Geographic distribution	IUCN Status
N. pygmaeus	pygmy slow loris	Cambodia, China, Laos, Vietnam	EN
N. javanicus	Javan slow loris	Indonesian island of Java	CR
N. bancanus	Sody's slow loris	Indonesian islands of Bangka and Belitung	CR
N. menagensis	Phillippine slow loris	Phillippines; Sabah and Sarawak Malaysia; Kalimantan, Indonesia; Brunei	VU
N. bengalensis	Bengal slow loris	Bhutan, Cambodia, China, India, Myanmar, Laos, Vietnam	EN
N. coucang	Sunda slow loris	Indonesia, Malaysia, Singapore	EN
N. kayan	Kayan slow loris	Kalimantan (East/South), Sarawak and Sabah Malaysia	VU
N. borneanus	Bornean slow loris	Indonesian island of Borneo (central/south)	VU
N. hilleri	Sumatran slow loris	Sumatra, Thailand	EN

1.5.2. FEEDING AND FORAGING BEHAVIOUR

Many species of mammal use forelimbs in arboreal locomotion, but primates are unique in that all species use hands for foraging and feeding (Peckre *et al.*, 2016). Haplorhines are classified as having precision grip, using various digit postures to grasp for food (Christel, 1993, Christel & Fragaszy, 2000; Pouydebat *et al.*, 2009), while strepsirrhines are known either to use the mouth to grasp fruit or a specialised whole-hand precision grip to catch insects, without individualisation of the fingers (Charles-Dominique, 1977; Napier, 1960,

1961; Petter, 1962; Scheumann *et al.*, 2011). Like other strepsirrhines, Lorisinae hands have a fairly fixed pattern of control, meaning that all fingers press the object toward the distal or proximal palmar pads (Bishop, 1964). Lorisines have a powerful whole-hand precision grip, with forceps-like design, applied during the capture of animal prey or whilst gripping branches during foraging behaviours (Bishop, 1962; MacNeilage, 1990; Napier, 1960), but far less so for dexterous manipulation of food items (Poindexter et al, 2018). Lorisines feed on a combination of exudates, animal products and arthropods to varying degrees (Nekaris, 2014; Starr *et al.*, 2008). In the wild, slow lorises have been observed to consume a diet comprised of nectar, gum and insects (Nekaris, 2014). This will be explored further in discerning how slow loris morphology limits or enable their ability to adapt to new and introduced food resources, from a conservation and evolutionary perspective.

1.5.3. THERMAL STRATEGY

Of the extant strepsirrhines, Lemuriformes have been a main focus for thermoregulation research in primates (Blanco *et al.*, 2018; Fietz & Dausmann, 2006; Dausmann, 2008, 2014; Dausmann *et al.*, 2005; Schmid, 2000; Schmid & Kappeler, 2005). In 2010, the first study to prove heterothermy in a wild non-lemur species was conducted, recording evidence of daily torpor use by *Galago moholi* in response to limited food resources and low ambient temperatures (Nowack *et al.* 2010, 2013). This study marked a new surge of interest by researchers in examining other species of Strepsirrhini, to understand the evolutionary paths of heterothermy in primates (Table 1.3).

Table 1.3. Described heterothermic primates and their expressed level(s) of heterothermy (hibernation, multiday torpor, daily torpor), body mass (g), habitat type and IUCN Red List classification; Moderated and updated from Dausmann & Warnecke (2016).

Family	Species	Heterothermy	Body mass	Habitat type	IUCN	Reference
Cheirogaleidae	*Cheirogaleus crossleyi*	Obligate hibernation	350	High-altitude rainforest	DD	Blanco & Rahalinarivo, 2010
Cheirogaleidae	*Cheirogaleus major*	Obligate hibernation	300	Littoral rainforest	DD	Lahann, 2007
Cheirogaleidae	*Cheirogaleus medius*	Obligate hibernation	130-250	Low-altitude dry forest	LC	Dausmann *et al.*, 2004, 2005
Cheirogaleidae	*Cheirogaleus sibreei*	Obligate hibernation	250	High-altitude rainforest	CR	Blanco *et al.*, 2013;
Cheirogaleidae	*Microcebus berthae*	Daily torpor	30	Dry forest	EN	Ortmann *et al.*, 1997; Schmid *et al.*, 2000
Cheirogaleidae	*Microcebus griseorufus*	Daily torpor; multiday torpor; Hibernation	50	Spiny forest	LC	Kobbe & Dausmann, 2009; Kobbe *et al.*, 2011
Cheirogaleidae	*Microcebus murinus*	Daily torpor; multiday torpor; Hibernation	58-70	Dry forest	LC	Schmid, 2001; Schmid & Speakman, 2000
Cheirogaleidae	*Microcebus ravelobensis*	Daily torpor	40-70	Dry forest	EN	Lovegrove *et al.*, 2014; Randrianambnina *et al.*, 2003
Cheirogaleidae	*Microcebus rufus*	Daily torpor, multiday torpor; Hibernation	40	Rainforest	VU	Ali & Huber, 2010; Randrianambinina *et al.*, 2003
Galagidae	*Galago moholi*	Daily torpor	90-250	Thornbush savannah	LC	Nowack *et al.*, 2010, 2013
Lorisidae	*Nycticebus pygmaeus*	Multiday torpor	420	Deciduous forest	VU	Ruf *et al.*, 2015

Lorisids have regularly displayed both behavioural and physiological reactions to low ambient temperatures, as well as numerous unique traits that suggest they may have a thermal strategy not so distant from their sister group, the galagids (Müller, 1975, 1985; Reinhardt *et al.*, 2016; Reinhardt *et al.*, 2019; Rode-Margono *et al.*, 2014; Ruf *et al.*, 2015; Starr *et al.*, 2012, 2013; Streicher, 2004; Whittow, 1977). *Nycticebus* have a unique fur coat, with a high insulation capacity (Müller, 1979; Streicher, 2004; Streicher *et al.*, 2017). Researchers observing wild *N. pygmaeus* populations in Northern Vietnam regularly detected seasonal pelage and colour change, as well as body weight variation, with animals reaching their maximum body weight during the cold winter months (Ratajszczak, 1998; Streicher 2004, 2005). Post-mortem examinations conducted by Streicher found intraabdominal fat reserves, suggesting *N. pygmaeus* store brown fat (brown adipose tissue), and is likely the cause for winter weight gain (2004, 2005). This is a physiological trait most common in hibernating species, and brought forth a strong inclination that *Nycticebus* could possibly attain heterothermic traits that merely were not observed due to their cryptic behaviour and understudied nature.

Prosimians have lower BMRs comparatively (to anthropoids), while the subfamily Lorisinae have historically displayed unusually low BMRs in both *Loris* and *Nycticebus* species (Müller, 1979, 1983, 1985; Nekaris, 2014; Wang *et al.*, 1995; Xiao *et al.*, 2009, 2010). In controlled environments, *Nycticebus* spp. have displayed marked drop in T_b below their TNZ in responses to cold temperatures, as well as the capacity to actively warm themselves over a short period of time (Eisentraut,1961; Müller, 1975, 1979; Ruf *et al.*, 2015; Whittow, 1977). One of the first studies to investigate the thermoregulation of a slow loris species (Sunda slow lorises *Nycticebus coucang*) recorded a resting MR that was consistently 40% lower than the normal mammalian standard (Kleiber's Law; 1961), with a thermoregulation

index of 25—33 ˚C (Müller 1975, 1979). Müller noted that *N. bengalensis* (identified as *N. coucang* during the study; Roos *et al.*, 2007; Somura *et al.*, 2012) would assume a heat conserving curled-up posture (like a huddle) when ambient temperatures dropped below their TNZ, as a form of behavioural thermoregulation. Similarly, captive studies conducted on *N. pygmaeus* in Kunming, China found slow lorises to display an average T_b of ~34˚C and a BMR of 67.3% of that expected by Klieber's Law (Wang *et al.*, 1995; Xiao *et al.*, 2009). Xiao *et al.*, (2010) conducted a similar study in Yunnan, China, where they recorded slow lorises to display a BMR of ~52% of that expected by Kleiber's Law, and a T_b with a lower thermoregulatory index (range: 32—37 ˚C). Differences in BMR recorded from these studies are likely due to different environmental conditions, where individuals of the same species have been known to acclimate their basal rates between ecosystems (Careau *et al.*, 2007; Müller & Diamond, 2001). These studies brought forth the first evidence that slow lorises attain the phylogenetic trait to lower their body temperature to conserve energy.

The Pygmy slow loris *N. pygmaeus* was observed to use multi-day torpor when ambient temperatures dropped below their TNZ in outdoor enclosures where they were exposed to the natural climate (Ruf *et al.*, 2015). This was the first study to quantify and confirm a species of Lorisinae to express the heterothermic phenotype. This study found captive *N. pygmaeus* individuals to display multi-day torpor, lasting up to nearly 63 hours in duration (T_b ranging: 11.0—29.9 ˚C), even when provided with a constant food supply (Ruf *et al.*, 2015). Individuals always entered torpidity at the end of their active period (late evening or early morning) and arousing most often in the afternoon, suggesting the use of active sun-basking or passive re-warming aided by heat from the sun (Ruf *et al.*, 2015). This was the first study to confirm heterothermy in a primate on continental Asia, which suggests that

heterothermy in primates may be a retained trait from mainland Africa, before the divergence of lorisids from galagids (sister group) to Eurasia during the geological formation of the *Gomphotherium* Land bridge. As lorises and galagos display similarities in their expression of heterothermy, researchers suggest that torpor is the more ancestral form of thermoregulation (Ruf *et al.*, 2015) where hibernation is a more derived trait, being only observed in the Malagasy primates (Blanco *et al.*, 2018). It is further hypothesised that the ability to express torpor in times of food scarcity facilitated the colonisation of the island of Madagascar by primates from Mainland Africa (Kappeler, 2000; Nowack & Dausmann, 2015).

1.5.4. WHY THE JAVAN SLOW LORIS?

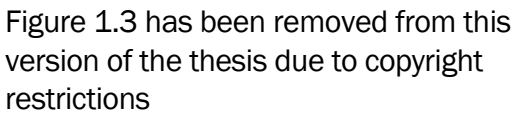


Figure 1.3. A scientific illustration of the Javan slow loris (*Nycticebus javanicus*) as displayed on the list of the 'World's Top 25 Most Endangered Primates'. Illustration by S Nash.

The Javan slow loris is endemic to the island of Java, Indonesia, with a body mass range of 850—1100 g (Rode-Margono *et al.*, 2014; Poindexter & Nekaris, 2017). Predominantly due to deforestation from anthropogenic land use and the illegal pet trade, *N. javanicus* is listed as Critically Endangered on the IUCN Red List, and is listed on Appendix I of CITES (Convention on the International Trade of Endangered Species). This species is also included on a list of the 'Top 25 Most Endangered Primates' (Figure 1.3; Mittermeier *et al.*, 2012, 2014, 2016). On the island of Java, less than 8% of forest remains due to agricultural and urban expansion, with the vast majority occurring at altitudes above 800 m asl (Nijman, 2013). While this species historically ranges in lowland forest, deforestation for agriculture means that in order to find suitable habitat, slow loris populations must move to higher elevations with a greater daily temperature range and less predictable resources (Cabana *et al.*, 2017; Nijman, 2013; Reinhardt *et al.*, 2016).

Nycticebus javanicus has been observed to perform long bouts of immobility during their active hours (including spontaneously falling out of trees), particularly in response to cold and high levels of luminosity, from moonlight (Rode-Margono & Nekaris, 2014). It has been reported numerous times that slow lorises have cold extremities (specifically hands) during capture and collaring procedures for field research (Reinhardt & Nekaris, pers obs. 2018). Additionally, Rode-Margono and Nekaris (2014) note an observation of one Javan slow loris displaying torpor, although there is no data reported. Due to this, the notably low metabolic rate and thermoregulatory index of other *Nycticebus* species recorded, I hypothesised that *N. javanicus* would display the heterothermic phenotype.

Nycticebus javanicus has been observed to consume a diet consisting of arthropods (whole), gum exudates, young leaves (bamboo shoots), angiosperm flowers and nectar, and

the mesocarps of fruits, at varying ratios between populations (Rode-Margono *et al.*, 2015; Moore *et al.*, 2012, Cabana *et al.*, 2017). The majority of angiosperm floral nectars present on the island of Java (where *N. javanicus* is endemic) are introduced across decades of agricultural practices (Macqueen, 1996; Riswan *et al.*, 1996; Thulin *et al.*, 1981*)*. These floral introductions would demand *Nycticebus* species to adapt to new food resources in order to maintain a stable dietary intake and their behavioural patterns.

While often regarded as solitary animals, extensive research has revealed Javan slow lorises to be fairly social. While individuals may forage in solitude, they spend more than half of their time in social proximity to another slow loris, engaging in behaviours such as grooming, playing, and feeding (Nekaris, 2014). In regards to mating, Javan slow lorises form stable uni-male uni-female social pairs with an individual that they produce offspring with, raise their young with, and on occasion, share a sleeping site with (Nekaris et al., 2017). While slow lorises have been observed to sleep in pairs, this is not a strict behaviour, and will often sleep alone. In these instances, an animal will curl into a ball for sleep, but not necessarily with their mate. When animals do display gregarious sleeping behaviour, it is no more than a mate and their shared offspring, displaying potential social thermoregulation behaviour.

Climate change trends and habitat loss demand wild animal populations to adjust, often beyond their thermal niche, on a multitude of levels over time. An understanding of endothermic flexibility in light of environmental change is becoming vital. *Nycticebus javanicus* must adapt their thermal strategy and behavioural patterns to cope with climatic variations outside of their thermoneutral zone. As this species largely ranges in human-modified landscapes, they are an ideal model to understand primate ecophysiology in the Anthropocene.

The objective of this dissertation was to determine the general ecophysiology of Javan slow lorises (*N. javanicus*), in understanding their physiological tolerances and behavioural adjustments within their natural environment—particularly in response to intra and inter-daily climatic and seasonal variations. I focus on factors that share a direct relationship with an animal's metabolism and energetic balance (Figure 1.4): resource acquisition, activity and resting patterns and temperature regulation.

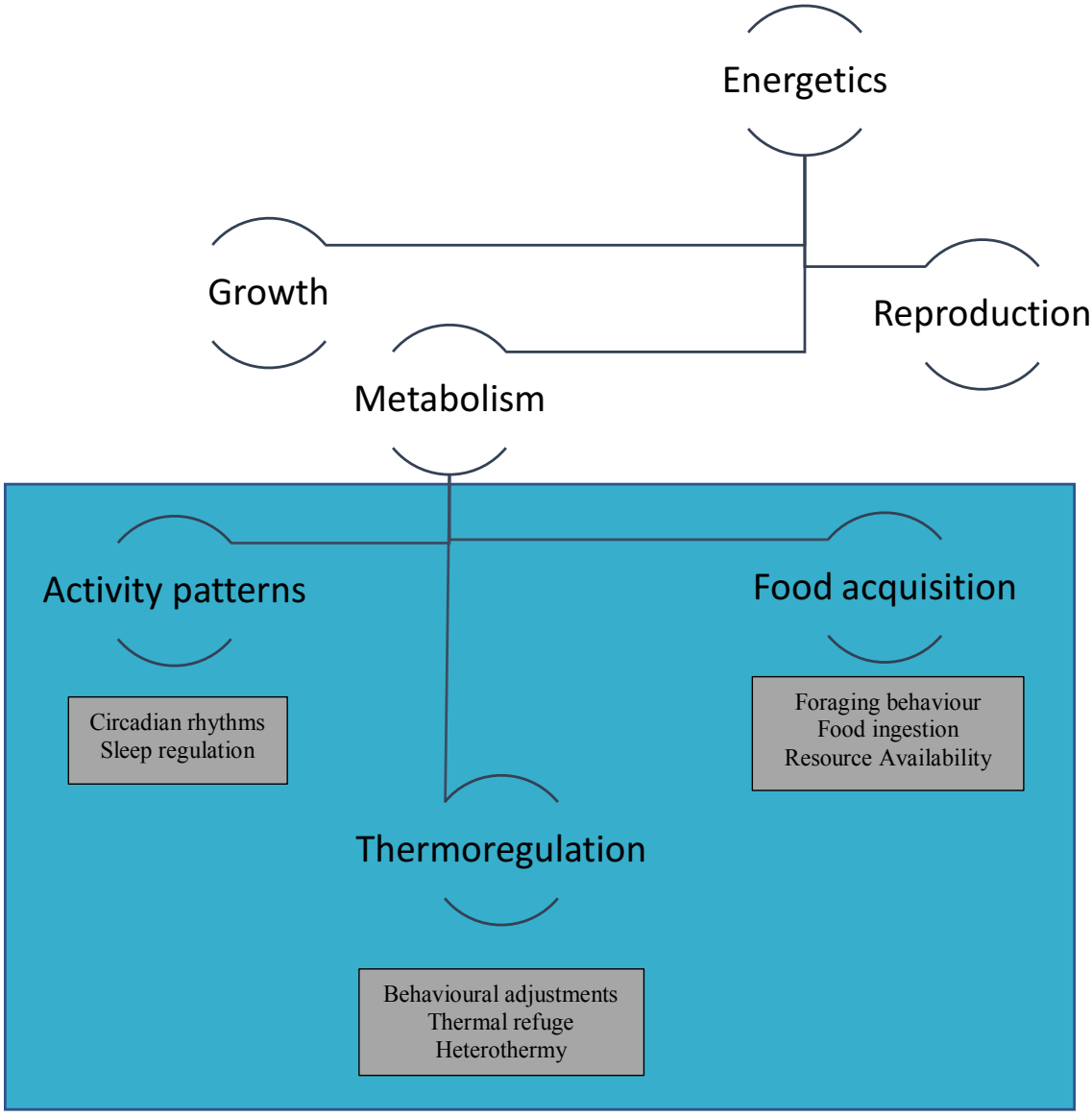


Figure 1.4. Thesis focus, in relation to various components in ecophysiology as defined by Tomasi & Horton (1992). The blue square represents factors immediately related to metabolism, and the topics that will be elaborated on throughout this thesis.

As the environment and an animal's physiological limitations are key drivers of an animals' energy expenditure, I ask the following questions to understand the relationship between Javan slow loris physiology and their environment:

1. What are the physiological tolerances of slow lorises on a thermoregulatory level, and how do they adjust to their environment?

2. How do slow lorises manage sleep patterns in response to external environmental influence, and intrinsic homeostatic sleep need?

3. How do slow lorises acquire food energy in a human-modified environment, on a behavioural level? And how does their behavioural ecology role fit into the ecosystem?

4. Can external bio-loggers serve as suitable (and least invasive) proxy methods in allowing us to measure sleep and thermoregulation in wild primates?

5. How can these results be applied to evidence-based conservation and future conservation management and planning for populations of *Nycticebus javanicus*?

To answer these questions, I used a combination of least-invasive bio-logging methods concomitant with nightly behavioural observations and ecological monitoring. I first focused on acquiring direct evidence of torpor expression in a wild *Nycticebus* population, through measuring temperature regulation in response to environmental changes (Chapter 3), followed by sleeping site selection in response to ambient temperatures and habitat availability (Chapter 4). Next, I examined their circadian rhythms in monitoring sleep, its regulation and relationship to daily activity patterns and environmental variation (Chapter 5). Lastly, I examined their behavioural flexibility in food acquisition, with a particular focus on floral nectar resources and its ecological role as an introduced non-native species (Chapter 6). In conclusion, I discuss the overall costs and benefits of these results in understanding the behavioural and physiological adaptations in balancing time and energetic demands of this species in a given environment. I also discuss how these results can aid in conservation efforts (Chapter 7), particularly towards methods in re-wilding (reintroductions, translocation, ecological substitutes) and managing wild populations, as well as their evolutionary implications and suggestions for future research.

Figure 2.1. The agroforest (where agriculture and forest overlap) landscape mosaic, where planted trees surround the agricultural area used in Cipaganti, West Java, at 1,350 m asl where Javan slow lorises range. Photo by: KD Reinhardt.

2.1. OVERVIEW

To investigate the research questions presented in this thesis, I used a cumulative dataset configured of data collected by myself and various other trained members of the Little Fireface Project (LFP) research team (as part of a long-term research project) between March 2015 and August 2018. I conducted fieldwork at the LFP research site in Cipaganti, West Java (Figure 2.1) between March 2015 and September 2016, after data collection was continued by trained research assistants until I returned to the site in March 2017 (Figure 2.2). This research was done in collaboration with two Indonesian institutes on the island of Java: Research Centre for Biology at the Indonesian Institute of Sciences (Biology-LIPI) and the Universitas Gadjah Mada (UGM). Together, we received approval for this research by the Indonesian Ministry of Science and Technology, RISTEK (research permit no. 1421/FRP/SM/VIII/2015), adhering to the legal requirements of the Indonesian government. All research was conducted in adherence to the ethical practice and guidelines provided by the Association of the Study of Animal Behaviour and were further approved by the Animal Ethics Sub-committee of Oxford Brookes University.

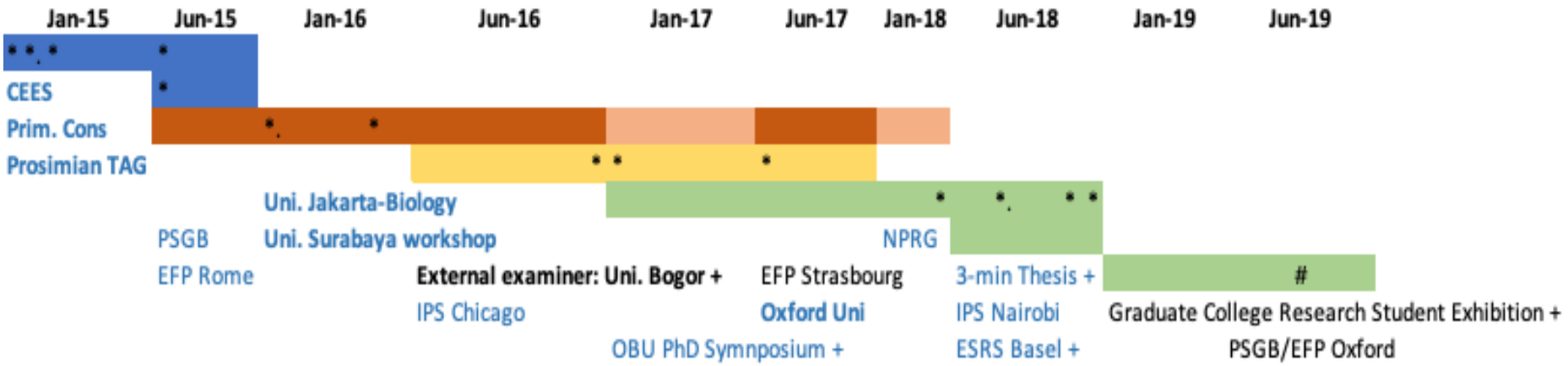


Figure 2.2. A timeline representation of the full PhD research process (4 years) from January 2015 until January 2019. Solid blue blocks represent project planning and preparation; orange blocks represent data collection (with faded blocks representing collection by research assistants); yellow blocks represent data management and analyses; green blocks represent writing of dissertation and manuscript drafts. Asterisks represent timing of dissemination of PhD research, with attended conferences/events listed in order below, in vertical columns. Blue titles represent events where I presented my PhD research, whereas black represents posters and non-PhD related presentations. Bold conference titles represent events where I was invited to speak, while the additional + symbol indicates events that I received an award/funding to attend. # indicated publication of work in this dissertation to peer-reviewed scientific journals.

2.2. STUDY AREA

LFP is located near the village of Cipaganti, Cisurupan in the Garut District of West Java, Indonesia (7°16'44.30" S, 107°46'7.80" E, 1200 m asl). LFP is an on-gong research project that was established in 2011, and has been based at this location since 2012 (Figure 2.3). This is the first ever long-term project on any wild nocturnal strepsirrhines (Nekaris, 2016). I chose to conduct my study via this project and location as it provides a habitat for a large population of *N. javanicus*, which is habituated and highly visible for relatively continuous monitoring due to its human-modified landscape. Collaborating with an on-going study allowed me to monitor focal animals in detail over multiple years.

Figure 2.3. The Indonesia islands, where the Little Fireface Project (LFP) field site is located in Cipaganti, Garut district, West Java, Indonesia (7°16'44.30" S, 107°46'7.80" E, 1200 m ASL). The LFP site is labelled with a yellow star.

2.2.1. ECOLOGY

Historically, the island of Java was once characterised by over 6,500 species of flora (including 4,500 native species) across a diverse number of forest types (van Steenis & Schippers-Lammerste, 1965; Whitten *et al.*, 1996). Due to centuries of agricultural expansion, as of 2005, an estimate of only 8% (1.2 million ha) of forest remained on Java, with the majority of that land occurring in montane regions over 800 m asl (Budi *et al.*, 2009; Nijman, 2013).

Cipaganti is located on Mount Puntang, which is a part of the Java-Bali Montane Rain Forests Ecoregion (Figure 2.4). Java has long ago been converted to an agroforestry system, where an estimated 70-80% of staple crops grown in Indonesia (maize, soybean, groundnut, tuber crops) are produced (Donner, 1987; Whitten, 1996). An agroforest is a human-modified habitat used by agriculturalists, where crops are grown in addition to non-agricultural trees and vegetation types (Rice & Greenberg, 2000). This habitat type characterises the region where LFP is stationed (and where slow lorises range), amongst a mosaic of traditional gardens mixed with an annual perennial rotating crop system—rows of crops, interspersed with rows of tall trees and patches of bamboo—used by local farmers (Nekaris *et al.*, 2017; Reinhardt *et al.*, 2016; Rode-Margono *et al.*, 2014). The area of Garut (where the LFP field site is based) is continuously listed amongst the most critical areas on Java and Bali that are supported by local agriculture (RePProT, 1989).

Figure 2.4. Top: A cabbage and carrot crop field with a property line of planted trees (including banana *Musa acuminata* and eucalyptus *Eucalyptus* spp.). Bottom: A photograph of Cipaganti from the roof of the LFP field station in Cipaganti, Cisurupan, West Java showing the proximity of the village to the mountains. Slow lorises range across the mountains and the agricultural landscape, where they share the area with humans, particularly farmers. Photos by KD Reinhardt.

Over the duration of LFP's established location in the Cipaganti area researchers have recorded all animal sightings during regular data collection (behavioural observations, vegetation surveys, line transects, group scans) both at night and during the day. Animals recorded to occur in the Cipaganti area besides Javan slow lorises include: bats (tailless fruit bat *Megaerops kusnotoi*, Javan pipistrelle bat *Pipistrellus javanicus*, Horseshoe bat *Rhinolophus* spp.); birds of prey (crested serpent eagle *Spilornis cheela*, Javan hawk eagle *Nisaetus bartelsi);* carnivores (Javan binturong *Arctictis binturong* ; Javan ferret badger *Melogale orientalis*; Javan leopard *Panthera pardus melas*; Javan palm civet *Paradoxurus javensis*, leopard cat *Prionailurus bengalensis*, small Indian civet *Viverricula indica*); and snakes (reticulated python *Python reticulatus*, Javan spitting cobra *Naja sputatrix).* In addition to wild species, domestic dogs *Canis familiaris*, feral cats *Felis catus* and humans *Homo sapiens* are regularly observed in the farmed areas. While *N. javanicus* in the only primate species observed in this habitat, langurs *Presbytis comata* and gibbons' *H. moloch* range on the opposite side of the mountain.

2.2.2. CLIMATE

The island of Java experiences a diverse variation in climate between regions and throughout the year, mainly deriving from air mass oscillations from the inter-tropical convergence zone (Whitten *et al.*, 1996). This climatic effect creates a more extreme variation between lowland and montane climates, causing temperature to decrease at an average of 0.6 °C for every increased 100 m asl (Braak, 1929; Nijman, 2013). Additionally, Java has experienced gradually harsher dry seasons with each consecutive El Niño event (since 1970), resulting in water shortage and higher frequency of forest fires, as well as lower crop yields (Whitten *et al.*, 1996).

The LFP research site is located in an agroclimate zone that is classified as 'permanently cool', for all land that is >1,000 m asl and a 'slightly seasonal' climate with experienced 1-3 dry months (wet: dry, 14:33%; RePPProT, 1990; Schmidt & Ferguson, 1951; Whitten, 1987). Cipaganti is a submontane environment near the equator, causing a larger range of ambient temperature across 24-h than would be experienced across seasons. The region of Cipaganti has been recorded to have daily ambient temperatures ranging from minimum lows of 10.4°C - 20.7°C to maximum highs of 26°C – 35.6°C (Reinhardt *et al.*, 2016, 2019), with strong winds during the months of January and February, and a high susceptibility to landslides occurring between April and August (Rode-Margono *et al.*, 2014).

2.3. DATA COLLECTION

2.3.1. BIOTELEMETRY

Bio-logging methods have greatly increased detectability in monitoring the behavioural ecology of *N. javanicus,* supporting long-term research conducted by LFP. At the time of this writing, 52 individuals had been fitted with 17 g VHF radiotransmitter collars (PIP3, Biotrack, Wareham, United Kingdom) since 2012. Radio-collars have been consistently used by the project to aid in individual identification, locating focals and observing their regular behaviours (Nekaris *et al.*, 2017). Individuals are routinely captured from trees by an experienced Indonesian researcher using protective gloves, where individuals were fitted with collars without use of anaesthesia. Capturing was done at a minimal frequency and on a rotating basis to replace radiotransmitter batteries (average 12—14 month battery life) to maintain individual identification.

If individuals displayed dispersal-like behaviour, collars were completely removed in the case the animals should disperse beyond our ability to monitor them regularly. During my time at the fieldsite, I regularly managed captures and the fitting of radio-collars (Figure 2.5). In collaboration with the field team, I collected morphological measurements and performed health checks opportunistically, during captures following the protocol in Poindexter & Nekaris (2017). The protocol for the capturing and collaring of these animals has been approved by the Animal Ethics Subcommittee stated above (See chapter Overview 2.1).

Figure 2.5. Top: KD Reinhardt weighing a Javan slow loris for opportunistic morphometric measurements; Bottom: KD Reinhardt fitting a Javan slow loris individual with a radio collar, with adhered data loggers; Photo by D Geerah.

SKIN TEMPERATURE

To measure the thermoregulation index of focal animals, I equipped 18 electronic skin temperature (T_{sk}) loggers (Stoehler Co., Hamburg, Germany) to the inside of radiotransmitter collars (Figure 2.6) of slow lorises using epoxy adhesive and heat-shrink (n=13 individuals; 5 adult males, 6 adult females, 1 juvenile male, 1 juvenile female). Tsk loggers were placed at the base of the collar, so the sensor would be in contact with the underneck, where slow lorises have thinner hair, ensuring better skin contact. Loggers recorded T_{sk} and stored data every 15-minutes, with a capacity for ~150 consecutive days. I removed and replaced loggers from individual's collars every ~120 days, as data can be lost when batteries die after ~150 days.

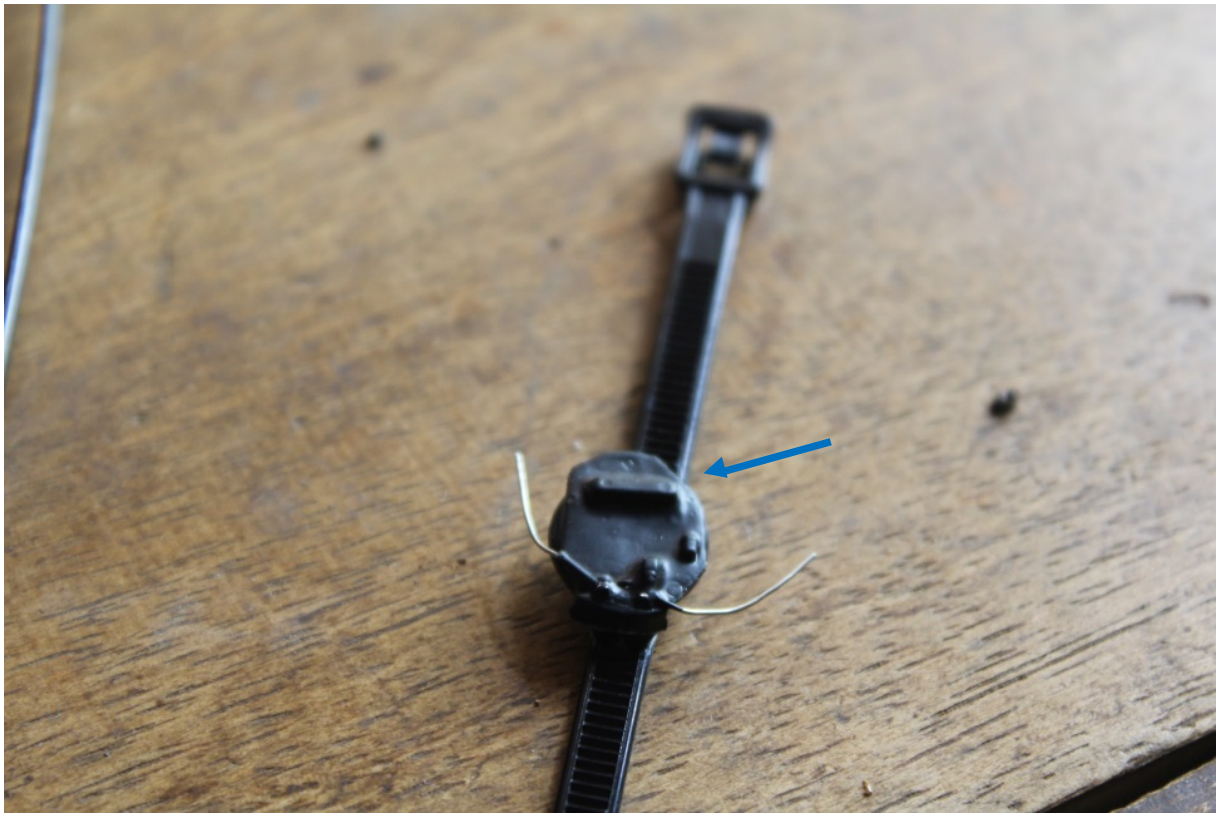


Figure 2.6. Method of attachment before launching skin temperature loggers. Before fitting loggers to individual Javan slow lorises, I would first adhere temperature loggers to the inside of the radio-collars (on zip-tie), then cover the zip-tie and the connecting wires with heat shrink. Note that the logger sensor is exposed (indicated with blue arrow), so that it can make direct contact with the animal's skin.

As *N. javanicus* is listed as Critically Endangered on the IUCN Red list, I chose to use least invasive bio-logging methods to measure T_{sk} as a proxy for T_b to investigate its thermal flexibility/limitations. When T_b cannot be measured due to its invasive methodology (i.e. surgical implants and anesthesia), T_{sk} is a reasonable proxy method for studying endangered species, where invasive research may be prohibited or considered unethical.

Geiser *et al.* (2004) hypothesised that reduction of MR in daily heterotherms is a reliant subsequent to an initial lowering of T_b. Following this hypothesis, it is suggested that lowering of T_b in daily heterotherms is presumption that MR is also being lowered, when temperatures drop below an animal's thermoneutral zone (suggestive of hypometabolism). Small animals show a lesser gradient between surface (cutaneous or subcutaneous) and core T_b, due to less distance between the two, as well as a higher thermal conductivity of body tissue (McNab, 1969; Brown & Bernard, 1991). Therefore, T_b can be inferred as a proxy to T_{sk}, when temperature-sensitive loggers are in contact with the skin, so long as atmospheric temperatures do not interfere (Audet & Thomas, 1996; Dausmann *et al.*, 2005).

ACCELEROMETRY

To record locomotor activity of focal animals, I attached accelerometer devices Actiwatch Mini: CamNtech Ltd., Cambridge, UK) to the VHF radio collars of slow lorises (n=14; 8 males, 6 females) as a proxy measurement to interpret activity levels. Before attaching to radiocollars, I programmed accelerometers to store full activity counts at 1-minute epochs using MotionWare software (CamNtech Ltd., Cambridge, UK). I attached accelerometers manually, using two small zip-ties (2.5 mm), where I locked one through the activity logger and the other through the first zip-tie and around the radio-collar cable (Figure 2.7). After

these were secure, I cut off the excess plastic and applied epoxy adhesive to the end of the cut. It is important to note that other types of glue (such as super glue) can degrade the plastic and allow the logger to fall off. If individuals required a new radio-collar, I mounted accelerometers prior to fitting the radio-collar to the animal, to both decrease capture time and ensure a secure attachment.

Actiwatch are 3-dimensional piezoelectric accelerometers manufactured by CamNTech and calibrated to human activity. These measurements are then accumulated at a set epoch, with the ideal interval being 1-minute (32 Hz frequency), where the data output is referred to as an 'activity score'. These measurements can be used to calculate or infer an animal's circadian rhythms, wakefulness, activity budgets and patterns, as well as energy expenditure or sleep (Jeanniard du Dot *et al.*, 2016; Reinhardt *et al.*, 2019; Sellers & Crompton, 2004). These loggers can store data at the capacity of the battery (standard lithium C2032); 1-sec intervals for 31 days, 1-min intervals for 91 days, etc.), and you can easily replace the battery in the field. Should the battery die while the logger is still attached to the animal, the data are stored and can still be retrieved at a later date. The use of accelerometers in wild primate research is limited to a few studies (Crofoot *et al.*, 2010; Jack *et al.*, 2010), with only a dozen being conducted on Strepsirrhines (Table 2.1). While there are other activity score-producing accelerometers, I here chose to use Actiwatch, as it is the most prominently used in primate research, making it most feasible for comparative research (Table 2.1).

Figure 2.7. Attachment method for accelerometers to radiotransmitters worn by slow lorises. Top: Placement location of accelerometers on the radio-collar with rubber heat shrink applied to protect animals from plastic edges; Bottom: Attachment of zip-ties, and removal of excess hanging plastic.

Table 2.1. A literature review of research conducted on strepsirrhine primates, using accelerometers. We include: the study species; accelerometer attachment method; environment type (wild, free-ranging or captive); the accelerometer manufacturer, and the model in parenthesis; the validation analyses used (supervised vs. unsupervised).

Study Species	Mounting method	Study environment	Accelerometer manufacturer	Validation	Reference
Eulemur mongoz	Harness	Captive	Custom-made	supervised	Sellers *et al.*, 1998
Eulemur f. fulvus	Collar	Wild	CamNtech (Actiwatch AW4)	unsupervised	Kappeler & Erkert, 2003
Varecia variegata rubra	Harness	Captive	Analog Devices (ADXL05)	supervised	Sellers & Crompton, 2004
Propithecus verreauxi	Collar	Wild	CamNtech (Actiwatch AW4)	unsupervised	Erkert & Kappeler, 2004
Aotus azarai	Collar	Wild	CamNtech (Actiwatch AW4)	unsupervised	Fernandez-Duque & Erkert, 2006
Aotus azarai	Collar	Wild	CamNtech (Actiwatch AW4)	unsupervised	Fernandez-Duque *et al.*, 2010
Propithecus verreauxi	Back mounted	Captive; free-ranging	Humotion (Munster, Germany)	Supervised	Wunderlich *et al.*, 2014
Hapalemur meridionalis	Collar	Wild	Advanced Telemetry Systems (ARC400)	Supervised	Eppley *et al.*, 2015
Cephalopachus bancanus borneanus	Collar	Free-ranging	Technosmart (AXY-3)	Supervised	Constantini *et al.*, 2017
Avahi meridionalis	Collar	Wild	Technosmart (Axi-3)	unsupervised	Campera *et al.*, 2019
Lepilemur fleuretae	Collar	wild	Technosmart (Axi-3)	Unsupervised	Campera *et al.*, 2019
Nycticebus javanicus	Collar-mounted	wild	CamNtech (Actiwatch-Mini)	unsupervised	Reinhardt et al, 2019 (Chapter 5)

Attachment methods of accelerometers to nonhuman primates have varied in both captive and wild studies, and between locomotion types. These methods include: collar-mounted accelerometers (Mann *et al.*, 2005; Papailiou *et al.*, 2008), accelerometers mounted onto jackets or custom-made harnesses (Golub *et al.*, 1999; Zhdanova *et al.*, 2002), glued to the subject's hair or inserted subcutaneously (Erkert *et al.*, 1986; Erkert, 1989). Researchers suggest the use of harness-mounted accelerometers for clinging and leaping species (Erkert & Kappeler, 2004; Sellers & Crompton, 2004). This is largely due to the fact that the use of radio-collars can be problematic for these animals, with the potential of being caught on a branch during regular locomotor activities. Alternatively, researchers have found radio-collars to be best suited for lorisids, in long-term individual identification and re-location (Nekaris, 2016). If wild lorisids are being radio-collared, the best method for using accelerometers is to mount the device to the radio-collar of the individual. Of course, if accelerometer data are collected in a captive setting, the device can be attached to one of the main types of collars available (non-radiotelemetry), usually made of rubber coated cable tie or tie wrap, leather, or metal.

Many studies have tested the long-term effects of bio-logger weight on wild animal behaviours, migration and reproductive success, suggesting the '5% rule', in regards to average body mass (Snijders, 2017). Furthermore, with many different biologging models being produced by different manufacturers, there is a clear difference in product weight, making it pertinent to calculate the percentage of body mass when selecting devices and mounting methods before attaching them to the focal animal(s). The combined weight of radio-collars (17 g), skin temperature loggers (<1 g; see above section) and accelerometers (7.7 g including 3 g battery) remained under the recommended 5% bio-logger weight limit

(Snijders, 2017), with adult *N. javanicus* individual body mass ranging between 850 and 1,100 g during the time of this study.

2.3.2. BEHAVIOURAL OBSERVATIONS

All collared slow lorises were followed on a regular rotating basis, with each individual being followed an average of two nights per month using instantaneous focal sampling at 5-minute intervals and *ad libitum* sampling (Altmann, 1974; Nekaris *et al.*, 2017; Rode-Margono *et al.*, 2014). The rota of individual focals were selected in attempt to balance monthly observations between sex (males and females), age (infant, juvenile, adult) and attaining a minimum of one focal observation on each individual per month. Following Poindexter & Nekaris (2017) and Nekaris *et al.* (2019) I used the following definition for age class: infant, birth until 153 days old; juveniles, 154-365 days old; subadults, 366-730 days old; adults, ≥ 731 days old.

Joined with the team of researchers at LFP, I identified and followed slow loris individuals each night using Sika receivers and Yagi antennae (Biotrack Ltd., Wareham, United Kingdom) and head torches with red filters (Cluson Engineering Ltd., Hampshire, United Kingdom). We collected a total 14,448 behavioural data points towards the cumulative dataset between March 2015 and August 2018. Behaviour was collected using a modified version of the behavioural ethogram used by Nekaris *et al.*, (2017). Each observation had a single focal animal that was followed by a team of two researchers. The data collected at each interval point included the individual's behaviour (Table 2.2), the plant species in use, the animal's height within the plant species (meters), their posture or locomotion, and proximity to conspecifics. When an individual was performing feeding or social behaviour,

we used instantaneous or *ad libitum* sampling to acquire more specific details on these behaviours (see section 6.2.2).

Table 2.2. Ethogram used to categorise behaviours observed by *Nycticebus javanicus* during nightly data collection at LFP.

Behaviour	Definition
Alert	Remaining stationary, but awake and seemingly vigilant, with active observation of the environment observer
Feeding	Ingesting food items
Foraging	Looking for food and investigating food resources without ingesting (visual and olfactory inspection)
Freeze	Interrupting locomotion and becoming motionless (maintained) in a rigid, alert posture
Groom	Autogrooming; using toothcomb or licking own fur
Rest	Remaining stationary, with eyes open (often hunched)
Sleep	Remaining stationary with eyes closed, in conjunction with sleep posture (head tucked forward between knees, like a ball)
Social	All interacting with conspecifics, including: • Play—performing friendly behaviours with conspecifics • Aggression—fighting between conspecifics, including biting, chasing and agonistic vocalisations • Allogroom—Using toothcomb and mouth to groom conspecifics fur
Travel	Engaging in continuous, directed locomotion from one location to another
Other	Unidentified behaviours that do not fit into the above definitions
Out of Sight	When the behaviour of animal not discernible, or when the location of individual is unknown
Eye Shine	When the behaviour of the animal is not discernible, but you can detect their location via their eye shine (including if they are active or inactive)

I followed individuals during their active hours, between 17:00 and 05:00 (Figure 2.8). When individuals were seen foraging on floral nectars, I collected continuous behavioural data. I used handheld GPS devices (GPSMAP 62s, Garmin Ltd., Olathe, KS, USA) to georeference each focal data point.

Figure 2.8. Top: KD Reinhardt collecting behavioural observations. Photo by F Cabana; Bottom: Field tracker Dendi and research assistant locating a *N. javanicus* individual using radiotransmitter and red filter head torches. Photo by W Tarniwan.

2.3.3. PHENOLOGY

I established permanent vegetation plots within the research area in 2014 that were maintained for the duration of this study (and continue to be monitored by the current team at the research site). I installed six Modified-Whittaker vegetation plots to monitor the phenology of food types consumed by *N. javanicus* as well as measure the habitat and species distribution in this unique habitat landscape. Modified-Whittaker plots, are nested vegetation sampling methods where vegetation sampling is collected in nested quadrats (subplots) of different sizes to avoid statistical complications, while controlling for the influence of plot size and shape on species richness reported (Stohlgren, 1995). These plots consist of: one 50 x 20 m plot (Plot D), serving as the full Modified-Whittaker plot perimeter; one inner central plot at 20 x 5 m (Plot C); two subplots of 5 x 2 m (Plot B) at diagonally opposing corners; ten subplots of 2 x 0.5 m (Plot A) dispersed evenly along the edges of the perimeter plot, and between subplots (Figure 2.9). The location of each perimeter plot was randomly selected using GPS points, omitting any points that fell within a farmer's crop field. I omitted these points as slow lorises do not use the crop fields themselves, so measuring the phenology of crops would serve no purpose in this study.

Modified-Whittaker plots have proven an effective method for ecological time series, such as monitoring long-term trends of phenology, while still allowing a robust analyses of species abundance for distinguishing native and non-native plant species (Stohlgren *et al.*, 1995; Yorks, 1989). Modified-Whittaker plots have also proven effective across various habitat types, from lowland tropical forest to semi-arid grasslands and shrubland (Ghorbani *et al.*, 2011; Campbell *et al.*, 2002), proving most suitable for future comparative research across the different species of Lorisinae, as well as continued research at LFP.

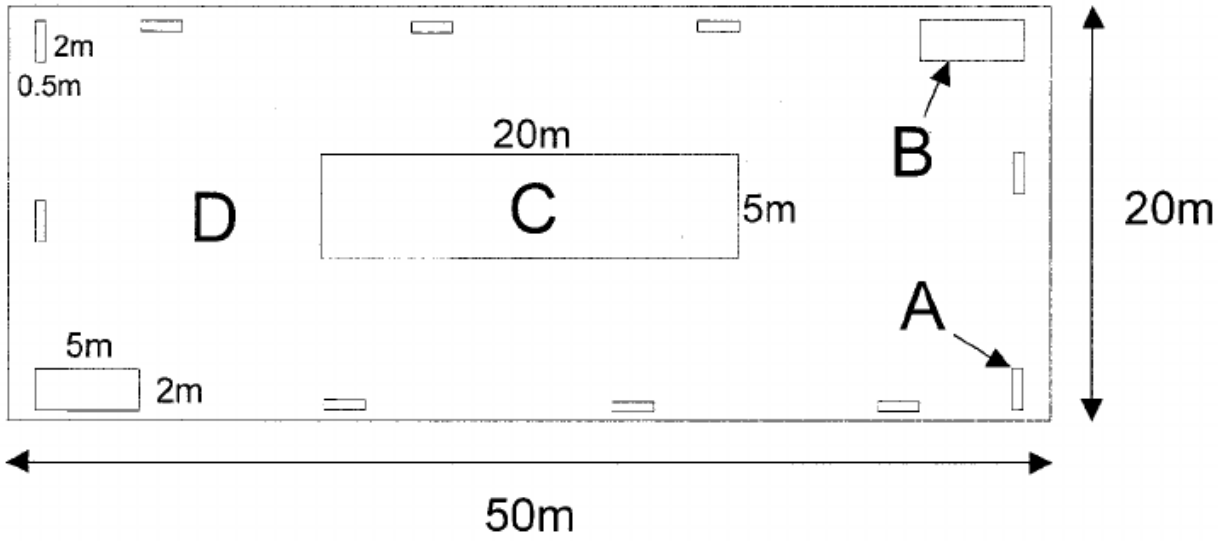


Figure 2.9. The design and dimensions used in setting up permanent Modified-Whitaker Plots, taken from Campbell et al, 2002. As permanent flags, we installed labelled wooden stakes with waterproof paint for each subplot, and regularly rotated biodegradable flagging tape on individual trees within each plot.

To estimate the spatiotemporal changes in food production of plant species that compose the slow loris diet, I monitored relative abundance of the plant parts (mature leaves, new leaves, flowers, fruit, gum) every two weeks, for each plant >1 m in height within the plots (Table 2.3).

Table 2.3. Plant species recorded in Cipaganti during establishment of modified-Whittaker plots in 2014. Species identification was conducted at the LFP research site during an herbarium survey with LIPI in early 2014. This list does not include crops planted by farmers that do not provide canopy (i.e. carrots, cabbage), as these have never been observed to be used by *N. javanicus*.

Family	Scientific name	Common name	Origin	Agricultural use
Caricaceae	*Carica papaya*	Papaya	SE Asia, Neotropics	Food
Cassuarinaceae	*Casuarina junghuhniana*	Forest oak	Indonesia	Timber, fuel, construction
Compositae	• *Ageratina riparia* • *Chromolaena odorata*	• Creeping croftonweed • Siam weed	• Central America, Caribbean • North America	• N/A • Traditional medicine
Ebenaceae	*Diospyros kaki*	Japanese persimmon	Southeast Asia	Food
Fabaceae	• *Acacia decurrens* • *Calliandra calothrysus* • *Calliandra tetragona*	• Green wattle • Fairy duster • Fairy duster	Australia	Animal fodder, timber, soil fertility
Lauraceae	• *Persea americana* • *Cinnamomum burmanni*	• Avocado • Indonesian cinnamon	• Central and South America • Southeast Asia	Food
Leguminosae	*Leucaena leucocephala*	Wild tamarind	Central America, Mexico	Fodder, compost
Meliaceae	*Toona sinensis*	Chinese mahogany	Eastern and southeastern Asia, North Korea	Timber, animal fodder
Moraceae	*Artocarpus heterophyllus*	Jackfruit	South Asia, Southeast Asia	Food
Musaceae	*Musa acuminata*	Banana	Indonesia	Food

Myrtaceae	*Eucalyptus sp.*	Eucalyptus	Australia	Traditional medicine, timber
Palmae	*Arenga pinnata*	Sugar palm	South Asia, southeast Asia, northern Australia	Food, traditional medicine
Poaceae	• *Dendrocalamus asper (besar)* • *Gigantochloa apus (tali))* • *Gigantochloa atter(temen)* • *Gigantochloa pseudoarundinacea (surat)*	• Giant bamboo • String bamboo • Sweet bamboo	Indonesia, Malaysia, India	Construction (housing, household items)
Proteaceae	*Grevillea robusta*	Silver oak	Eastern Australia	Construction
Rhamnaceae	*Maesopsis eminii*	Umbrella tree	Tropical Africa	Timber
Rubiaceae	*Coffea arabica*	Mountain coffee	East Africa	Food
Solanaceae	*Cestrum aurantiacum*	Orange cestrum	N/A	N/A
Theaceae	• *Camellia sinensis* • *Schima wallichii*	• Tea • Needlewood	Asia (east, south, southeast)	• Food • Construction (furniture, flooring)

While standard ecology limits measurements to trees >10 m in diameter at breast height (DBH) (Chapman *et al.*, 1994) we set no DBH limit, because the slow lorises are regularly observed to feed on terminal branches of plants. Phenology measurements were recorded using a relative scale modified from Meyer (1998; see Table 2.4). For *Calliandra* plants, seed pods (classified as fruits) were assessed on a scale of ripeness and dispersal. We also noted any anthropogenic changes to the plots (e.g. cutting down of trees).

Table 2.4. Phenology scale used to assess biweekly temporal abundance of plant products (flower, gum, fruit, leaves) from food resources of the home-ranges of the study population.

Code	Flowers
0	None visible
1	New buds, limited to a few branches (<50% of tree complement)
2	Newly bloomed flowers, limited to a few branches (<50% of tree complement)
3	Fully bloomed flowers; full complement (>50%)
4	Few bloomed/wilting flowers, limited to a few branches (<50% of tree complement). *** Presence of flowers on the ground***
	Gum
0	None visible
1	Scarce gum visible; gum in viscous droplets
2	Gum visible, in coagulated, hard clumps
3	Gum visibly flowing, from many large, gouged holes
	Fruit
0	None visible
1	Few new fruits on a few branches; unripe
2	Abundance of ripe fruits in the tree
3	Few ripe fruits on a few branches
	Fruit on Ground
0	None visible
1	1-2 ripe fruits on the ground, around the base of the tree
2	More than 2 ripe fruits on the ground, around the base of the tree
3	1-2 unripe fruits around the tree
0	None visible
	Mature Leaves
1	Matured leaves, visible on few branches (< 50%)
2	Full complement of leaves on the tree (>50%)
3	Few matured leaves, because they are dried and falling off the tree (< 50%)
	New Leaves
0	None visible
1	Few newly sprouted leaves, visible on few branches (< 50%)
2	Full complement of leaves on the tree (> 50%)
3	Few new leaves visible, because they are maturing or matured (< 50%)

2.3.4. MONITORING CLIMATE AND WEATHER

HOBO data loggers are the most frequently used climate sensor loggers to measure microclimates in field ecology (Bramer *et al.*, 2018), making them most suitable for comparative research. A microclimate can occur within a few meters of canopy cover, between vegetation types or even within specific structural parts of a plant (e.g., stamina or petal) causing temperatures to deviate as much as 20 °C (Dietrich and Korner, 2014; Suggitt *et al.*, 2011). Therefore, determining an animal's response to its immediate climate proves difficult through larger analyses (e.g. climate envelope models) where ecologist use data collected from meteorologist stations, demanding measurements to be made at a much smaller scale. A microclimate can display varying climate variation from the atmosphere on a temporary timescale, requiring monitoring of variables at hourly intervals, or shorter (Bramer *et al.*, 2018).

To monitor climatic variation over time, I randomly placed climate station loggers (Hobo U23-002) within each social group's home range to collect measurements of ambient temperature (T_a) and percentage of relative humidity (rh%) at 15-minute intervals with an accuracy of ±0.5°C (Figure 2.10). Loggers were placed on the nearest tree branch at 5-6 m height, by climbing the tree and adhering the logger to a branch using string and flagging tape. Each individual logger was georeferenced for retrieval of data, and distinction between slow loris home ranges.

Figure 2.10. A HOBO U23-001 climate logger installed on a tree branch, out of direct sunlight. Photo by KD Reinhardt.

2.3.5. FOOD SAMPLES

During the time of this study, other researchers were investigating various aspects of the feeding ecology of this population of *N. javanicus*. Dr. F Cabana, a then PhD student, was measuring and quantifying the nutritional content of the *N. javanicus diet, with a focus on tree gum*, whilst a researcher (CA Henry) measured and quantified insect abundance within the home ranges of *N. javanicus* social pairs. To complete the full dietary components of *N. javanicus* in this area, I focused on the feeding of floral nectars and its availability and phenology in Cipaganti.

Measurements of sugar volume and energy value of plant nectars is regularly performed in ecological studies to understand plant-animal interactions, such as energetic rewards for various pollinators, and visitor attractants and preference (Dungan *et al.*, 2004; Morrant *et al.*, 2008). Various methods can be used to extract nectar samples from floral parts in the field, including centrifuging flowers, rinsing flowers, micro-syringe use or micro-capillary action (Morrant *et al.*, 2008; Swanson & Shuel, 1950). If a centrifuge is accessible, flowers containing nectar samples could be plucked from their plant and placed face down into test tubes, where the centrifugal force should extract the nectars into the base of the test tube (Swanson & Shuel, 1950). This method can result in some excess contents from the flower/petals if centrifuged for too long, but is quite time efficient if potential contamination of additional flower parts is not a major concern with the analyses. Nectar can also be collected by washing out flowers with distilled water and collect all of the run-off (Mallick, 2000). The sample must then be evaporated to omit excess water. For flowers yielding particularly low quantities of nectar per flower, the best suited methods include filter paper blotting (McKenna & Thomson, 1988; Kearns & Inouye, 1993) and microcapillary use (Morrant *et al.*, 2008; Harder & Cruzan, 1990; Southwick *et al.*, 1981).

Calliandra flowers contain such small quantities of nectar, 25 µL micro-capillary action and filter papers were best suited to extracting nectar directly from the plants (Figure 2.11). Each flower on the plant holds relatively low quantities of nectar, often requiring a few hundred flowers to extract enough for a 100 µL sample, or filter papers blotted with many flowers from a single plant. For microcapillary samples, once 100 µL of nectar was acquired from an individual plant, samples were mixed with 70% ethanol and stored in the fridge for up to 2 weeks. Filter papers containing nectar samples were stored in petri dishes until they dried, after which they were transported to envelopes, and labelled with sample details

(date and time, corresponding slow loris home range; plant species; sample type(nectar or pollen)). Unfortunately, the nectar samples collected could not be analysed for nutritional content in determining energetic rewards, due to equipment failure in the collaborative local lab, in Bogor, West Java.

Figure 2.11. Top: KD Reinhardt collecting pollen and nectar samples from *Calliandra calothrysus*. Photo by Aconk Ahmad; Bottom: Photograph of *C. calothrysus* seed pods post dispersal, and pre-collection. Photo by KD Reinhardt.

As LFP is a long-term research project, I was able to use the project's full dataset where applicable to further investigate my research questions. This involved sorting and organizing large datasets into cohesive and useable formats for each analysis, merging the cumulative dataset with additional biologger data (climate variables, T_{sk}, accelerometers, GPS, behaviour) and phenology and habitat data that I collected in conjunction with the LFP research team. To manage these datasets of diverse variables and intervals, I used a combination of Microsoft Excel v16.16.5, tab delimited text files, Garmin Basecamp, Ranges, ArcGIS v10.4, SPSS v24 , R statistics v 3.6.0 and R Studio, and MathWorks Simulink and MATLAB v2018a (Table 2.5).

Over the course of my PhD, I developed skills in multiple software programmes and analyses. I used SPSS to test variables for normal distribution, and testing of auto-correlation between covariates to be in the same statistical model/test using the Spearman's rank correlation test; I used R (a free software that can compute a broad range of analyses from simple statistics to complicated graphics) to sort and run generalized linear mixed models (GLMM) on torpor expression (Chapter 3), linear mixed-effect model (LMM) on behavioural thermoregulation (Chapter 4), and behavioural flexibility (Chapter 6); I used MATLAB (a powerful software used to develop algorithms and create mathematical models) to visualize continuous data, specifically temperature data and actigraphy. I also used this software to perform time series analyses and data simulation tests (Chapter 5). I present all mean values ± SD (standard deviation) or as mean ± SEM (standard error of the mean) when comparing all individuals, with justification for each in chapters 3 through 6. Individual analyses are described further in each chapter. Significance was set to 0.05 for all statistical analyses.

Table 2.5. Summary of statistical analyses run for each chapter, and the software used to perform them.

Chapter	Topic	Analyses	Software / Programme
3	Heterothermy Torpor expression	• Descriptive statistics • T-test • Wilcoxon Signed Ranks Test • Generalized Linear Mixed Model	• R 3.6.0: package lme4, car, ggplot • MATLAB R2018a
4	Sleeping site selection Thermoregulation Microclimate use	• Resource Selection Functions • Logistic regression • MCP's • Linear Mixed-Effect Model • Mann-Whitney U test	• ArcGIS v10.4 • Ranges • SPSS v24 • R 3.6.0: packages lme4, MuMIn, ggplot
5	Quantification of sleep Circadian rhythms	• Survival Curves • Time-Series Analysis • Repeated Measures ANOVA • Wilcoxon Signed Ranks Test • Data simulation	• MATLAB R2018a and Simulink
6	Plant phenology Foraging behaviour Activity budgets and resource availability	• Descriptive statistics • Chi-square Association test • Mann-Whitney U test • Friedman test	• R 3.6.0: packages geom_smoot, stats

Figure 3.1. An adult female Javan slow loris (Ena) at the Little Fireface Project field site, in a huddled posture on a terminal *Calliandra* spp. branch. Photo by A Walmsley.

Variations and shifts in climate influence endothermic animals in a multitude of ways. These include: population range shifts (Fordham *et al.*, 2013), habitat structure and use (Korstjens, 2010; Overdorff, 1996), phenotypic and evolutionary responses (Manne, 2013; Cross *et al.*, 2013; Austin *et al.*, 2012) and behavioural and physiological adaptations (Adolph, 1990; Dausmann & Warnecke, 2016; Donati *et al.*, 2011; Sato, 2012). Endothermic animals use means of heat production to maintain their core body temperature (T_b) and homeostasis. In order to survive environmental changes where normothermy is not solely sufficient, endothermic animals are additionally capable of low expressions of hypometabolism (metabolic reduction: lowering the T_b by ~2 °C and metabolic rate by ~20%; Heldmaier *et al.*, 2004).

Mammalian species vary in their thermal flexibility; where some are thermally specialised and can maintain their T_b by means of increased energy expenditure or behavioural thermoregulation, others are capable of prolonged periods of hypometabolism (such as torpor or hibernation). Mammals capable of torpor and hibernation use are heterothermic—endothermic animals with the capacity to lower their metabolic rate (MR) and T_b well below the above stated threshold of hypometabolism observed in strict endotherms (Heldmaier *et al.*, 2004; Lyman & Chattfield, 1955). This physiological process is a method of energy and water conservation, most often observed in species inhabiting harsh environments where animals must cope with seasonal stressors (such as scarce food resources) and climatic shifts (Carey *et al.*, 2003; Geiser, 1988; Heldmaier, 1989; Heldmaier *et al.*, 2004; Humphries *et al.*, 2002; Nowack *et al.*, 2013).

Certain environmental conditions may have buffered the need to express or engage in hypometabolism, while a given species could indeed possess the genotype (Calisi & Bentley, 2009; Soto *et al.* 2017). While heterothermy is most often associated with small animals that must cope in temperate and arctic environments, researchers are starting to discover that more species in other environment types are capable of expressing this phenotype (Geiser, 2013). Climate change trends show an increased variation in seasonality, particularly in tropical environments, where we see higher ambient temperatures, more intense seasonal variation and more frequent climatic events (Lovegrove *et al.*, 2013; Wright *et al.*, 2009). Such environmental changes have led to the discovery of more species that express torpor and hibernation use, revealing more heterothermic taxa (Geiser & Ruf, 2015; Nowack *et al.*, 2010; Nowack *et al.*, 2015; Ruf *et al.*, 2015; Streicher *et al.*, 2017). This leads to new questions regarding our understanding of the evolution of heterothermy, and the need to reassess its evolutionary path.

The majority of physiological studies having been conducted in: captive settings where environmental conditions and food resources are controlled (e.g. *Mus* sp., Soto *et al.*, 2017; eastern chipmunks *Tamias triatus*, Scott & Fisher, 1970); free-ranging captive settings where animals are exposed to natural climatic fluctuations, but still maintain a constant food supply (e.g. eastern grey squirrel *Sciurus carolinensis*, Periera *et al.*, 2002; pygmy slow loris *Nycticebus pygmaeus*, Ruf *et al.*, 2015); or semi-captive settings, where wild animals are removed from their natural environment (usually a tree hole or burrow), and placed in artificial hibernacula, with controlled environmental factors (e.g. western fat-tailed dwarf lemur *Cheirogaleus medius*, Blanco *et al.*, 2013, 2016). Due to recent advances in biologging technology and field research, physiological research has progressed in the wild,

providing new insights to ecophysiology and the expression of heterothermic phenotypes in response to various natural environments (Geiser, 2013).

In the Order Primates, only a few species have been observed to display the heterothermic phenotype, all categorised within the Infraorder Strepsirrhini (see section 1.5.1). In 2015, Ruf *et al.* recorded evidence of the most recently discovered heterothermic primate species, the pygmy slow loris *N. pygmaeus,* displaying seasonal use of multi-day torpor in northern Vietnam. This was the first study to provide data beyond anecdotal observations that heterothermy is expressed by an Asian primate, as well as the first study to quantify torpor in lorisines (Blanco *et al.*, 2018; Streicher *et al.*, 2017). While providing insight to this species' physiological capabilities, this study was conducted in a semi-controlled environment, limiting our understanding of the species (and potentially the genus) regular physiological activities in response to seasonal food resource availability and the environment. Nevertheless, this discovery demanded a reassessment of our understanding of the evolutionary path of heterothermy in primates.

I hypothesised that *N. javanicus* would express levels of heterothermy in the wild, in tropical environments with a wide range of annual climatic variation. Using light-weight biologgers (see section 2.3.1) to measure the slow lorises skin temperature in order to test this hypothesis, I asked the following questions: i) Does *N. javanicus* display thermoregulatory control?; ii) Does *N. javanicus* thermo-conform to ambient temperatures in their environment?; iii) If *N. javanicus* displays levels of heterothermy, are they correlated to seasonal phenology of food resources or climatic factors?; vi) Does expression of thermoregulation differ between sexes, age categories, or weight? I investigated if any of these responses vary over time, to understand population-level adaptations. I discuss these

results in the context of heterothermic evolution, and the origins of this adaptive physiological trait in the Order Primates.

3.2. MATERIALS AND METHODS

3.2.1. SKIN TEMPERATURE

I attached T_{sk} loggers to the inside of radiotransmitter collars of slow lorises (see section 2.3.1: Capture and Collaring) between June 2015 and April 2017 (N=1,102 days; Figure 3.2).

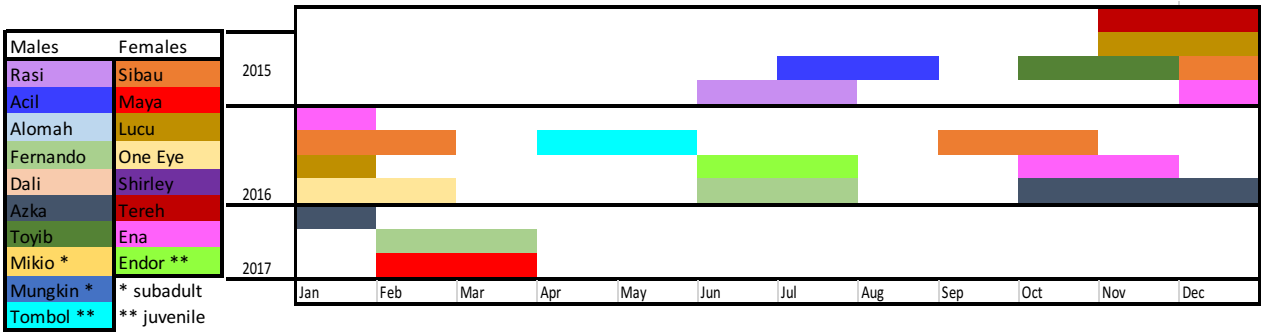


Figure 3.2. A timeline of skin temperature logger employment between individuals. Each colour represents a unique individual (see legend).

3.2.2. CLIMATE AND FOOD RESOURCES

To determine the influence of climate and food availability on slow loris thermoregulation index, I regularly monitored climate variables (section 2.3.4) and phenology of food resources (section 2.3.3) observed to be consumed by slow lorises at this site, including the duration in which T_{sk} measurements were recorded.

3.2.3. STATISTICAL ANALYSES

In previous studies, comparison of T_b and T_{sk} showed linear relationships, although T_a is found to influence T_{sk}, particularly when T_a drops below T_b (Audet & Thomas, 1996; Dausmann, 2005). By only using data while animals are resting, one can bypass the possibilities of movement causing ambient temperature to contact the T_{sk} sensor, that would skew the temperature measurements (Dausmann, 2005). Additionally, by the animal positioning itself into their typical sleep posture, the T_{sk} logger is positioned closest to the chest, providing the most accurate proxy for T_b. I define torpor following criteria of Willis (2007), where T_{sk} falls below the threshold of 33°C for more than 2 hours during a period of suppressed activity.

I ran t-test analyses to test if expression of thermoregulation varied between sex (male, female), as well as age groups (adult, juvenile). I additionally ran these tests to compare expressions of torpor with presence of gum, and presence of nectar in each individual's home range. I ran Wilcoxon Signed Ranks tests to confirm t-test results were not biased due to assumpotion violations, as T_{sk} was not normally distributed. To compare T_{sk} with body mass, I ran linear regression analyses.

To test if torpor expressions were correlated to seasonal phenology of food resources or climatic factors, I fitted torpor data to a GLMM using minimum T_{sk} recorded during torpor bouts as the response variable. Predictors selected for the model included T_a, gum availability, nectar availability, body mass (g), and sex (male, female), using slow loris individuals as random effect. I tested variables for multicoliearity, and used likelihood-ratio tests to compare the full model against a corresponding null model with random (individual) and control (T_a) variables.

3.3. RESULTS

Of the skin temperature loggers set up, only twelve loggers from eleven individuals (five adult males, four adult females, one juvenile male, one juvenile female) were retrieved with data. T_{sk} loggers stored temperature measurements at 15-min intervals for 31 ± 26.3 days on each animal (collective 279 days; Figure 3.3).

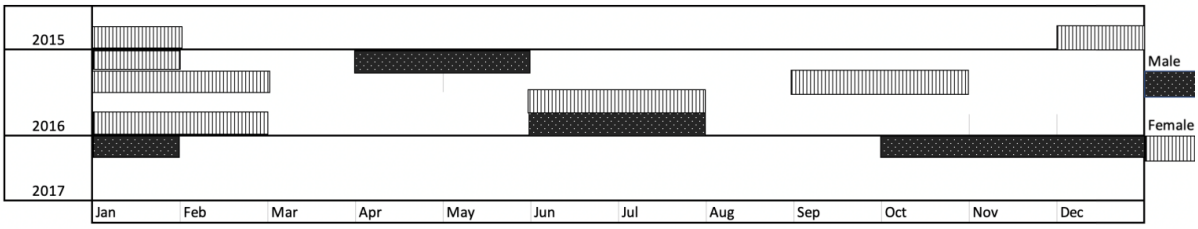


Figure 3.3. Timeline of retrieved Tsk recordings on individual animals (n=11) between 2015 and 2017, shown separately for males and females. Each rectangular block represents one individual slow loris.

Of the eleven slow lorises, all individuals displayed sporadic torpor bouts during all months of the year, ranging from 2.5 to 11.8 hours in duration. The mean and median T_{sk} remained in normothermia, with most individuals remaining around 34.1 ± 1.3℃. All individuals were capable of lowering T_{sk} to a minimum of 20.5℃, with some going as low as 14℃ beyond 2-h durations, suggesting all individuals are capable of entering a prolonged state of hypometabolism, categorizing them as heterothermic (Table 3.1). Slow lorises displayed a clear upper limit in T_{sk}, never exceeding 39℃. The average T_{sk} did not vary significantly between male and female slow lorises (t = -1.757, df= 19865, P = 0.079). Adults displayed a minimum T_{sk} threshold of one degree lower (14 ℃) during torpor expressions than juveniles (t= -2.15, df= 7770, P = 0.031).

Table 3.1. Thermoregulation index of individual slow lorises (n= 11), their age category, sex (m= male; f= female), average body mass, thermal flexibility (T_{sk} range), mean (± SD) and median T_{sk}, as well as the sample size (in days) for logger recordings.

Individual	Age	Sex	Average Body Mass (g)	T_{sk} range (°C)	Mean; Median T_{sk}	Battery life (days)
AC	Adult	m	1,013 ± 115.9 (n= 2)	15 - 39	31.0 ± 3.3; 31.5	24
AZ	Adult	m	905.8 ± 55.5 (n= 4)	16—35	30.8 ± 2.8; 31	15
ED	Juvenile	f	491 (n= 1)	17 – 38	31.4 ± 3.8; 32.5	98
EN	Adult	f	775 ± 124.7 (n= 6)	20.5 – 37.5	30.8 ± 4.1; 33	43
FE	Adult	m	972.8 ± 23.3 (n= 4)	14 – 36	31.4 ± 28.2; 32	30
LU	Adult	f	958.25 ± 57.3 (n= 4)	18.5 – 36	30.8 ± 4.1; 32.5	15
OE	Adult	f	960 ± 66.9 (n= 2)	16 – 38	22.7 ± 4.0; 22.5	15
RA	Adult	m	922 ± 82.5 (n= 3)	18 - 39	31.1 ± 4.0; 33	15
ST	Adult	f	1,028 ± 53 (n= 4)	15 – 38.5	30.9 ± 2.2; 31	31
TM	Juvenile	f	698 ± 144.2 (n= 2)	15 – 37.6	27.6 ± 5.2; 27.3	32
TO	Adult	m	1,090 ± 93.3 (n= 2)	23 - 36	31.5 ± 3.7; 33	30

I used linear regression analyses to test if the average body weight (g) of slow lorises significantly predicted the T_{sk} expressions of individuals. All regression analyses found body weight to be insignificant in predicting T_{sk} index, and explained <5% of the T_{sk} variance of T_{mean} (R^2= 0.02, df= 9, P= 0.69), T_{min} (R^2= 0.04, df= 9, P= 0.85), T_{median} (R^2= 0.19, df= 9, P= 0.75) expressed by slow lorises (Figure 3.4). Body weight explained 16% of maximum T_{sk} expressed by slow lorises, with a slight negative trend, although this was also insignificant (R^2= 0.16, df= 9, P= 0.23).

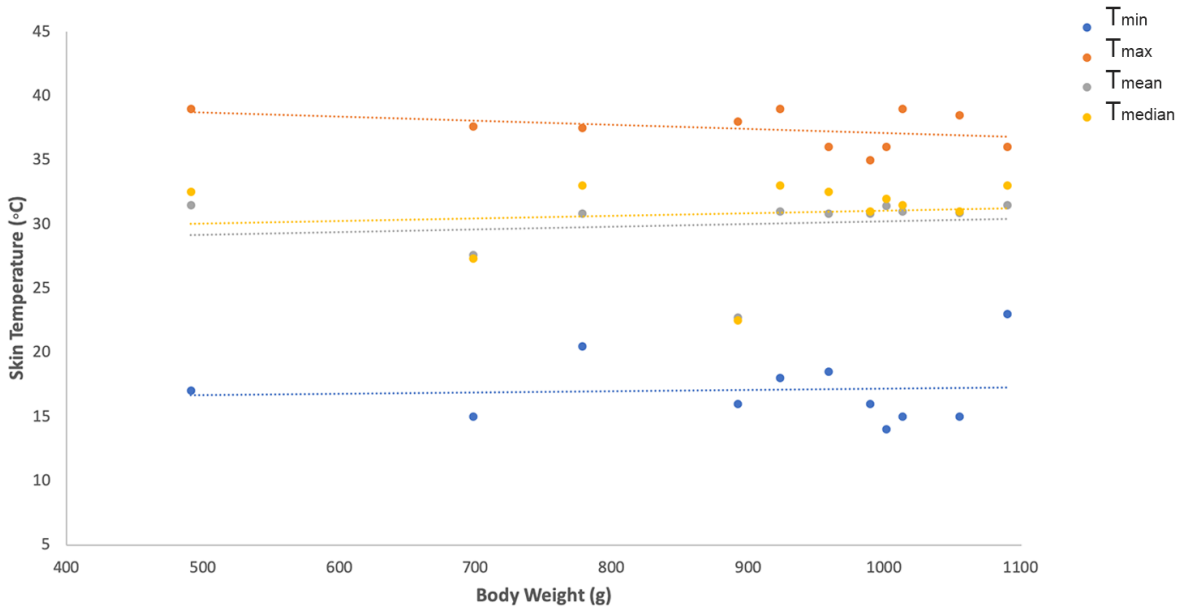


Figure 3.4. Relationship between body weight and T_{sk} ranges (minimum T_{sk}: $_{sk\_min}$; mean T_{sk}: T_{mean}; maximum T_{sk}: T_{max}) of slow lorises across the duration individuals wore T_{sk} loggers.

Javan slow lorises displayed an average torpor bout duration of 8.8 ± 4.43 hours, with an average T_{sk} expression of 20.9+ 3.7°C throughout a torpor bout (R^2= 0.21, df= 1, P< 0.001), where body mass predicts T_{sk} index. The deeper torpor bout observed were expressed by individuals with body weight closer to 900 g, measured at the time of capture and collaring when biologgers were fitted to the radio collar, and juveniles displaying a smaller range of expressed T_{sk} (Figure 3.5).

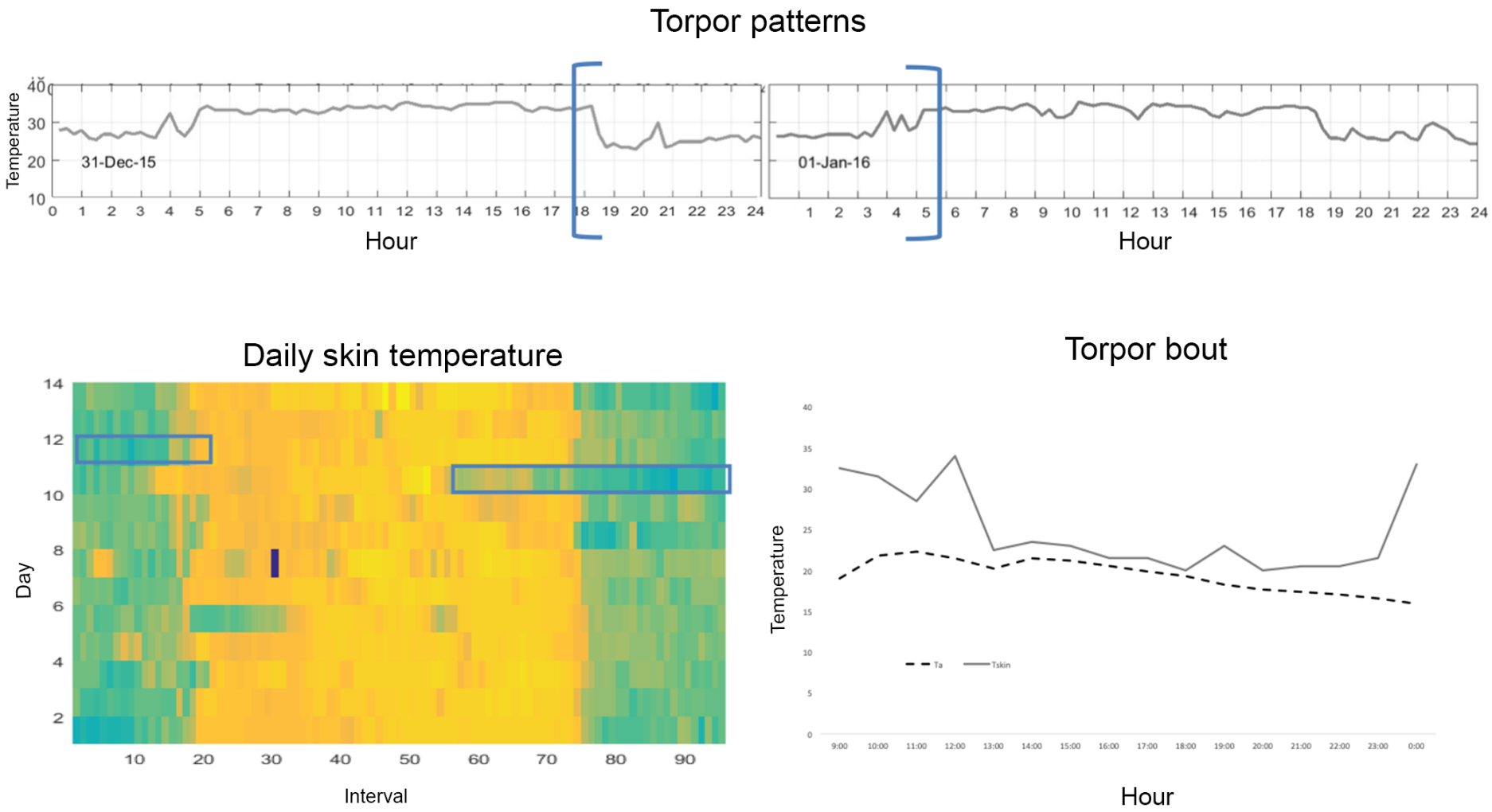


Figure 3.5. Top: a focus on the torpor patterns of the above selection from the top left figure (blue brackets) where EN expressed a torpor bout for ~9 hours, between the hours of 18:20 and 05:00. Bottom Left: A 3-D color gradient of daily T_{sk} patterns for individual EN over the duration of 14 days, where orange represents temperatures >30°C and blue represents <25°C. Bottom Right: A zoom in on a strict torpor bout, pre-suppression and post arousal, over the course of 9 hours in relation to ambient temperature.

Comparing the entire population as a whole, note that *N. javanicus* displays greatest T_{sk} flexibility between the months of July and September, with both the minimum T_{sk} and maximum T_{sk} expressed during the time of this study (Table 3.2; Figure 3.6). Slow lorises expressed an average T_{sk} of 28 ºC, and a minimum T_{sk} of 14 ºC when gum was less available as a resource. These were significantly lower compared to months when gum in more available (t= 31.258, df = 29283, P< 0.001) when individuals displayed an average T_{sk} of 30.4 ºC, and a minimum T_{sk} of 18 ºC.

Table 3.2. Monthly ambient temperature range, and expressed skin temperature ranges and median expressed across Javan slow lorises in Cipaganti, West Java. Data was collected between the months of December 2015 and January 2017. *N* = number of slow loris individuals sampled per month.

Month	T_a Range	*N*	T_{sk} range	T_{sk} median
January	15 – 25.6	5	18 – 38	30.05
February	17.3 – 22.8	2	21 – 37	30.92
March	15.5 – 25.2	2	22 – 35.5	30.99
April	14.7 – 29.9	1	15 – 37.6	27.63
May	16.2 – 26.7	1	21 – 37.5	30.03
June	14.2 – 26	2	21 – 37.5	32.12
July	11.5 – 27	2	14 - 39	27.71
September	12.8 – 27.3	1	14 - 40	30.50
October	13.9 – 29.7	2	15 - 39	31.5
December	13.5 – 24.8	2	16 – 38.5	25.85

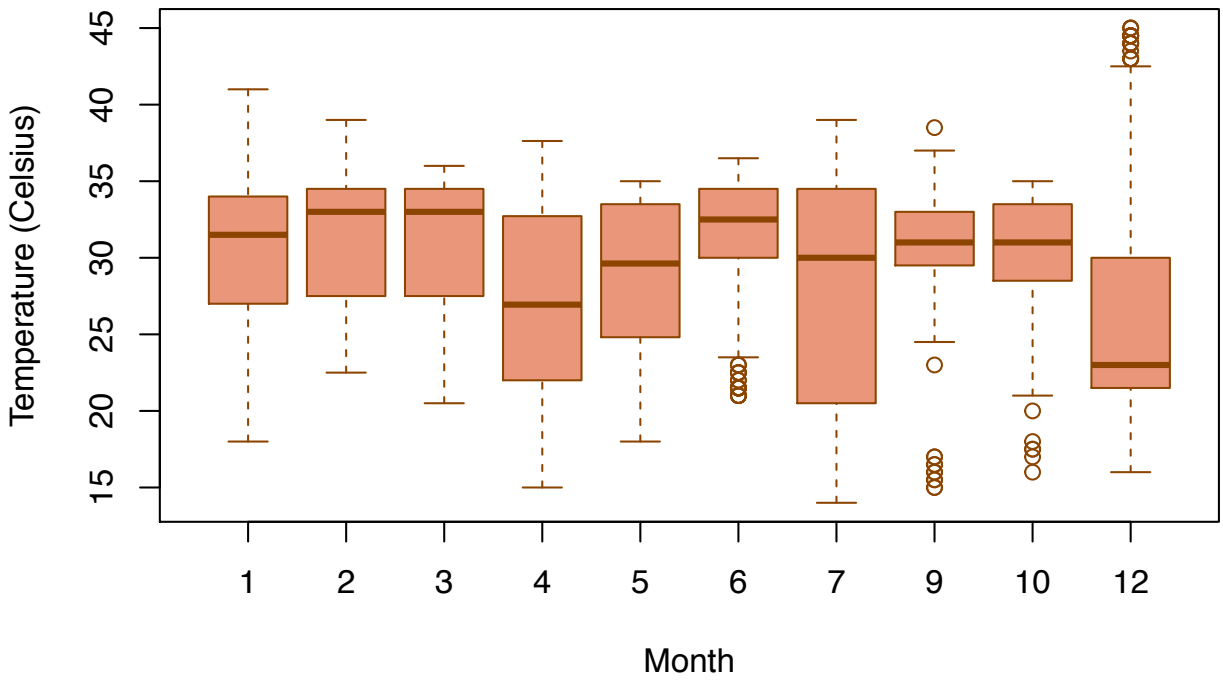


Figure 3.6. Box plots of skin temperature data collected from Javan slow lorises across months where individuals had loggers on. Solid brown lines represent the median value, and orange boxes represent the lower and upper quartiles. Dotted lines represent standard deviation from the mean. Open brown circles represent outliers of the data for each month.

Likelihood ratio tests indicated the GLM model for predicting minimum T_{sk} during torpor was significant (χ^2 = 44.45, df = 6, P< 0.001). The probability of slow lorises entering a deeper torpor (determined by lowering of T_{sk}) increased with changes in T_a and decreased with body mass (Table 3.3). The GLMM did not find any significant effect of the other predictors in the model for determining depth of torpor, which included presence of food resource availability (gum, nectar) and sex. Age was removed from the original model, as this was directly correlated to body mass (g).

Table 3.3. Results of the GLMM testing whether environmental (resource availability; T_a) and biological factors (sex, body mass) influence the minimum T_{sk} of slow lorises engaging torpor. Full null model comparison: $\chi^2 = 34.81$, df = 6, $P < 0.001$

Predictor variable	Estimate	SE	*P*
(Intercept)	28.930	1.458	
Gum	0.006	0.121	0.637
Nectar	-1.148	0.456	0.190
Sex	-1.504	1.921	0.305
Body Mass (g) *	-1.470	0.210	0.016
T_a **	1.660	0.426	<0.001

*Significance < 0.005; ** Significance <0.001

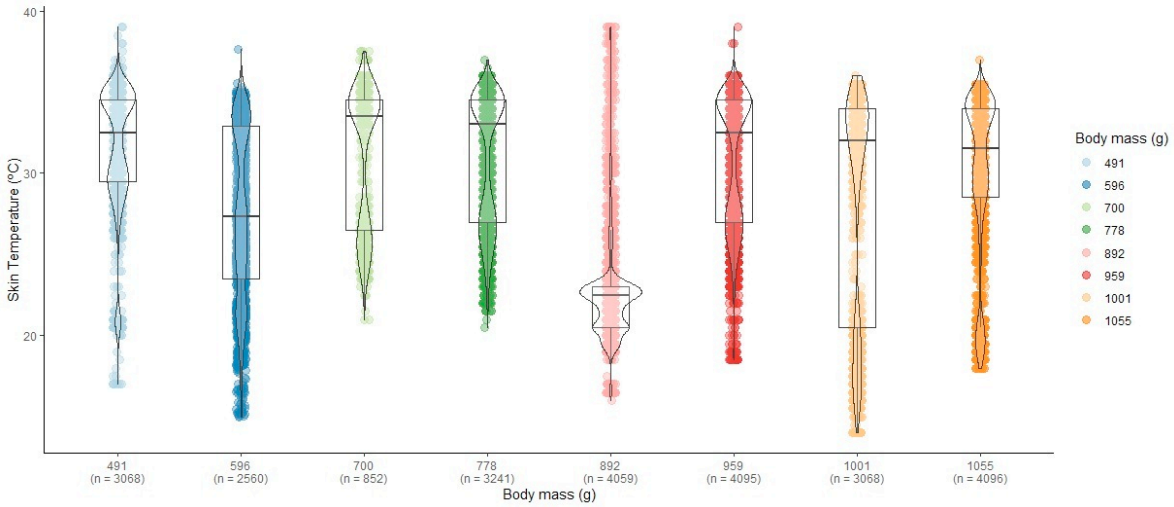


Figure 3.7. Probability of slow loris individuals expressing a lower T_{sk} during torpor as a function of body mass (g). Standard Error (SE). Each plot represents a different body mass recorded of animals fitted with skin temperature loggers.

3.4 DISCUSSION

Javan slow lorises displayed regular use of torpor, ranging from 2.5-11.8 hours in duration, a ~ 20°C thermal index range, and a mean T_{sk} normalcy at 34 ±1.3°C. While there is seemingly no difference between torpor expression between males and females, larger individuals were recorded to display deeper bouts of torpor. The clear body mass variation between younger and older individuals, however, cannot be overlooked.

Captive research has found many daily heterotherms to shift their thermoneutral zone to lower degrees during food deprivation, to cope with decreased energy (Kobbe et al., 2004; Soto *et al.*, 2017). *Nycticebus javanicus*, however, does not exhibit these same anatomical traits. Alternatively, this species has been observed in multiple populations to feed heavily on the floral nectars of fairy dusters *Calliandra calothrysus*, a leguminous tree that is commonly used in agricultural intercropping. The nectars of this species make up one-third of the *N. javanicus* diet, and are high in sugars (Cabana *et al.*, 2017). This fast energy intake may allow individuals to decrease the need for a more prolonged metabolic suppression, as we can see *N. javanicus* entered much deeper and longer torpor bouts during the months of April and July, when there are no *C. calothrysus* flowers in bloom. Alternatively, April was a dry month, with the coldest recorded ambient temperatures throughout the duration of this study, as well as the greatest range of ambient temperatures between night and day. Mice and hamsters studied in captivity can be induced into torpor via either reduced temperatures or caloric deprivation (Vyazovskiy *et al.*, 2014), suggesting both factors play a significant role in the phenotypic expression of torpor.

The relationship between MR and T_b during hypometabolism is suggested to vary between animals that express periodic torpor (daily heterotherms) and those that express multiday torpor (hibernators), where MR reduction varies between an animal's body mass, thermal capacity and level of torpidity expressed (Lyman *et al.*, 1982). Smaller animals are expected to more readily enter a state of torpor, due to an increase in surface area and therefore, a greater loss of body heat (Geiser, 2013; Geiser & Ruf; Lyman, 1972). Notably, however, *N. javanicus* is the largest recorded daily heterotherm in the Order Primates, with the maximum body mass recorded at 1,081 g (adult male, AZ) compared to the second largest recorded heterothermic primate being *N. pygmaeus* at a maximum recorded body weight of 600g (in captivity during the winter) (Schmid *et al.*, 2000). This indeed defies the comparative body mass correlation proposed by Ruf & Geiser (2015), in relation to all other heterothermic species (not including hibernators).

Microhabitat use, behavioural and postural thermoregulation are supplementary methods used by many heterothermic species to maintain and conserve heat during rest in conjunction with physiological thermoregulation (Bright & Morris, 1991; Buck *et al.*, 1991; Masing *et al.*, 2007; Mueller, 1999). Postural thermoregulation most often involves resting with the head curled under the abdomen, to keep the heart warmer than the body while still allowing blood flow to the abdomen (Lyman *et al.*, 1982). In this resting posture, the back is usually exposed to insulating material of a nest, or back of a tree hole or burrow. The combination of insulating materials with this resting posture aids as a buffer against external stimuli (e.g. from sudden changes in ambient temperatures) that could cause abrupt arousal while in this vulnerable state of torpor (Lyman *et al.*, 1982). Javan slow lorises regularly rest and sleep in a curled-up posture (often referred to as schlafkügel, or 'sleeping ball' in literature) resembling the stereotypical resting posture of other

heterotherms (e.g. common dormouse *Muscardinus avellanarius*, arctic ground squirrel *Spermophilus parryi*, Brandt's bat *Myotis brandtii*, western fat-tailed dwarf lemur *Cheirogaleus medius*), which may similarly aid as a buffer against stimuli that could evoke arousal during torpor bouts. While this postural behaviour likely aids in the process of heat production and maintenance of homeostasis, quantifying its influence proves difficult, as they exclusively sleep in this posture (thus, with no comparative posture). This population has also been observed to select bamboo stands as their sleeping sites >90% of the time (Nekaris *et al.*, 2017). Bamboo stands have been found to display a microclimate buffer against ambient temperature shifts, that may serve a similar function to tree holes or burrows as seen used by other strepsirrhines (Kappeler, 1998; see Chapter 4).

Research on mice has found housing conditions within the animals thermoneutral zone to 'blunt" or buffer the phenotype in obese mice—meaning, the phenotype has no need to be expressed in these conditions, but does not mean the animal does not have the genotype in which provides the capability to use this mechanism (Soto *et al.*, 2017; Vyazovskiy *et al.*, 2017). This phenotypic expression in conjunction with recent climate change trends could well describe the recent surge in discovering more animals capable of torpor and hibernation in the wild. Early captive studies on other slow loris species (e.g. the Sunda slow loris *N. coucang*) have found levels of endothermic thermal flexibility deviating from the normal mammalian limitations (25 – 37°C) as well as a low metabolic rate (Müller, 1979). During this study, we have recorded *N. javanicus* to express regular use of torpor in response to its natural environmental conditions. This particular population of *N. javanicus* ranges in a submontane region, where daily ambient temperatures and humidity vary substantially as altitude increases. These climatic conditions, therefore, could be a main driver in the expression of the phenotype. With more studies on the thermal flexibility of *N.*

javanicus at lower altitudes, we could further understand at which climatic conditions this phenotype is triggered. Examining these data, we suggest the importance of understanding thermal flexibility of mammalian species and populations in the wild to understand their physiological limitations to climate change and how we can apply this to conservation management plans. Furthermore, we suggest more species of the lorisids family be tested for heterothermic traits.

3.5 CHAPTER SUMMARY

In this chapter I showed data that suggests *N. javanicus* is heterothermic, expressing short bouts of torpor annually, both in relation to changing ambient temperatures and body mass. *Nycticebus javanicus* average T_{sk} was most influenced by ambient temperature and presence of food resources. *Nycticebus javanicus* displayed a greater T_{sk} range during the colder, dry months, when floral nectars of *Calliandra calothrysus* were not in bloom, providing lower resource abundance. Individuals varied in their thermal flexibility, with animals displaying a thermal flexibility ranging between 14°C and 39°C. All individuals displayed a clear maximum limit of T_{sk} that is less flexible, suggesting they are less capable of adapting to warming climates. In the next chapter, I discuss behavioural thermoregulation and selecting sleeping sites that provide thermal refuge against environmental changes, that might aid in decreasing the energetic costs of temperature regulation to maintain normothermia.

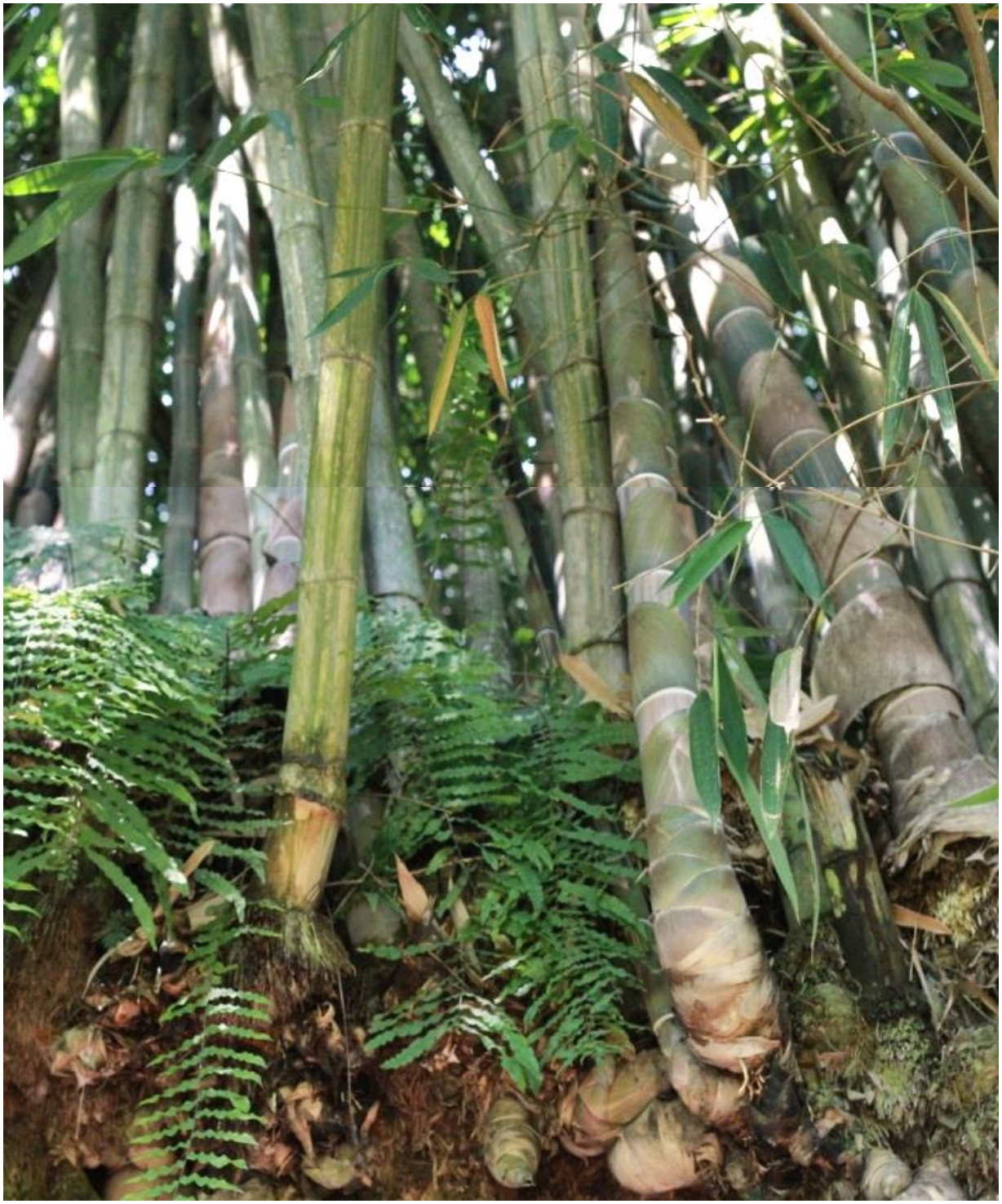


Figure 4.1. A bamboo cluster (including *Gigantochloa apus, G. atter, G. pseudoarundinacea*) growing in Cipaganti, West Java, at 1,350 m asl within the home-range of Javan slow lorises. Photo by KD Reinhardt.

4.1 INTRODUCTION

Bamboo is an evergreen woody grass in the subfamily Bambusoideae (family Poaceae), of high ecological importance. Both lowland and highland bamboo forest have been found to encompass a high diversity of taxa, including birds (e.g. spotted bamboo wren *Psilorhamphus guttatus*, bamboo foliage gleaner *Anabazenops dorsalis*), snakes (e.g. canebrake rattlesnake *Crotalus horridus*, Indian bamboo pit viper *Trimeresurus gramineus*), many species of rodent (e.g. tree shrews *Tupaia glis*, *Rattus* spp.) and large mammals (e.g. mountain bongos *Tragelaphus eurycerus*) (Bystriakova *et al.*, 2003, 2004; Bystriakova & Kapos, 2006; Sileshi & Nath, 2017). While many species inhabit bamboo forests, the use of bamboo as a resource is less common. Some mammals feed on bamboo (shoots and leaves) as part of a specialised diet, such as red pandas *Ailurus fulgens* (Johnson *et al.*, 1988) and giant pandas *Ailuropoda melanoleuca* (Nie *et al.*, 2015; Schaller *et al.*, 1985), and various species of primate, such as bamboo lemurs *Hapalemur* spp. / *Prolemur simus* (Tan 1999; Eppley *et al.* 2017; Eronen *et al.* 2017), Bale monkeys *Chlorocebus djamdjamensis* (Mekonnen *et al.* 2010) and golden monkeys *Cercopithecus mitis kandti* (Twinomugisha *et al.* 2003). Mountain gorillas *Gorilla beringei* in the Virunga Mountains do not frequently use bamboo forest, but will migrate to neighbouring high-elevation bamboo forests to seasonally forage on young bamboo shoots (Mahaney *et al.* 1995; Grueter *et al.* 2016). Similarly, both African *Loxodonta africana* and Asian elephants *Elephas mamixus* forage for food in bamboo forests (English *et al.* 2014). Bamboo thickets have also been observed to be used as sleeping sites by species of nocturnal primate, including owl monkeys *Aotus* spp. (Napier & Napier 1967; Wright, 1978,1981; Aquino & Encarnacion, 1986) and slow lorises *Nycticebus* spp. (Nekaris, 2014; Nekaris *et al.*, 2017; Svensson *et al.*, 2018).

A single bamboo plant is comprised of multiple culms (individual jointed stems of bamboo grasses, above ground) that grow in individual stands (the grouping of multiple culms from a single bamboo rhizome and root). Bamboo stands often grow in tightly formed patches of one or multiple species, called clumpings (Lobovikov 2007). Each individual culm of a bamboo stand maintains a constant core temperature because the centre of a culm is hollow, and its leaves and bamboo fibres block solar radiation (Campanello *et al.* 2007). Thus, if bamboo provides heat retention and shade, it could provide shelter and thermoregulatory benefits for animals. Due to their clumping and entanglement, bamboo plants provide an abundance of escape cover (Wiles, 1981) for sleeping animals and thus offer antipredation benefits.

The monsoon-belt of Southeast Asia and southern China hosts the highest biodiversity of bamboo and species richness (Bystriakova, 2003; Ohrnberger 1999). Indonesia alone has 450 species of bamboo that are considered of conservation importance, 56 of which are naturally occurring (Bystriakova 2003). It has been hypothesized that the sleeping sites used by slow lorises *Nycticebus* spp. aid as coping strategies against harsh environments and predation (Nekaris 2014; Nekaris *et al.* 2017), although this has never been tested. One species, the Critically Endangered Javan slow loris *N. javanicus* has been observed to frequently sleep in bamboo in West Java, Indonesia (Nekaris *et al.* 2017; Voskamp *et al.* 2014). I aimed to test the hypotheses that slow lorises select sleeping sites in order to shelter from the environment and to avoid predation using *N. javanicus* as a model, by testing what factors most influence the selection of bamboo stands as a sleeping site (see section 1.2.1). I predicted that bamboo sleeping sites would: i.) posess characteristics that offer protection against predation; ii.) shelter against climatic variables, displaying a

distinct microclimate to atmospheric temperatures; iii.) aid in thermoregulation, when ambient temperatures diverge from the species' thermoneutral zone.

4.2 MATERIALS AND METHODS

4.2.1. SLEEPING SITE SELECTION

Sleeping sites providing a unique microclimate may categorise as microrefugia, further protecting *N. javanicus* during rest (Bird *et al.*, 2002; Gillingham *et al.*, 2012a; Porter & Gates, 1969; Trivedi *et al.*, 2008). I defined a sleeping site as the vegetation structure where a slow loris settled for their daytime rest in the typical sleeping ball posture (c.f. Nekaris *et al.*, 2017), and the sleeping place as the height of the animal within the vegetation. I located daytime sleeping sites between the hours of 08:00 and 13:00 using the same radio-tracking methods as during nightly behavioural observations. I used thermal camera imaging (Flir Systems, Inc., Wilsonville, OR, USA) to aid in locating the individual's sleeping place within the sleeping site. For each sleeping site where I could see the individual, I recorded the plant species the animal was sleeping on, the height of the sleeping site and the individual's sleeping place, and the number of escape routes (determined by the number of connecting branches/trunks; Figure 4.2). Using handheld GPS devices (GPSMAP 62s, Garmin Ltd., Olathe, KS, USA) I GIS referenced each sleeping site. I recorded sleeping site locations weekly. Using this data and the cumulative LFP dataset, I focused on sleeping sites between July 2015 and January 2017.

Figure 4.2. A female Javan slow loris individual (Lucu; indicated by a blue arrow) in their sleeping place within a bamboo stand sleeping site (*Gigantochloa atter*). Photo by KD Reinhardt.

Over the entire LFP cumulative dataset, no animals in the area have been observed to be potential predators of *N. javanicus*. Researchers suggest nocturnal activity patterns combined with small body size decrease detectability by predators, making it a strategy of predator avoidance in itself. An explanation for lack of observed predation sightings could be the large population of humans that reside in the village of Cipaganti, as human presence alone has been observed to decrease predation by wild animals (Isbell, 1994). However, lack of evidence for predation does not dismiss predation as a selective pressure in sleeping site selection, and is likely to be an ultimate evolutionary pressure (Coleman and Hill, 2014). Nevertheless, while nocturnal carnivores are not known to predate on *N. javanicus*, they have been observed to use bamboo for foraging, shelter and sleeping sites, and so, I instead focus on their cohabiting role and bamboo use. To determine microhabitat

niche of bamboo use between slow lorises and small carnivores, I plotted all occurrences of other animals using bamboo plants, their observed behaviour, and the height of the animal's place within the bamboo.

Animals vary in their use of the environment, from landscape use to selection of specific flora species to microhabitat use (i.e. remaining in tree canopies, or terrestrial use only). Ecologists have devised various methods for measuring what resources animals use within their ecosystem. One sampling method, used-unused surveying, measures what resources an animal is directly observed to use, and assumes all else is unused in their regular, daily behaviours. This leaves error of assumption, as the used resources are known with certainty, while unused is not (Johnson *et al.* 2006). Long-term research regularly finds animals to perform variations in life-history traits and behaviours than were previously understood from studies of shorter duration (Chivers, 2013; Kappeler *et al.*, 2012; Nakagawa *et al.*, 2010; Reinhardt & Nekaris, 2016; Southwick *et al.*, 1986), including a broader use of resources (Eppley *et al.*, 2016). With this understanding, assuming unobserved use of a resource type means 'unused' is problematic, for a short-term study. Alternatively, use-availability sampling offers similar measurements of information, while avoiding the asymmetry of assumption, and merely interpreting unobserved use of a resource as 'available'. This allows an unbiased assessment of resource units within a habitat. Available resource units can be either used or unused by an animal, allowing a more practical assessment of habitat use.

Used-availability sampling was conducted on randomly selected vegetation plots (n=366) between April and August 2017. Each plot had a 5 m radius; the center mark of each plot was determined by the location of the *N. javanicus* individual. For ech used sample, three

randomly selected GPS coordinates were located to determine availability sampling, as control plots following Fortin *et al.* (2005). For each plot, I recorded the vegetation species at the center mark, the vegetation height, diameter at breast height (DBH), the canopy diameter and average canopy cover of the plot. The average canopy cover was calculated on a percentile scale for each direction (NESW) from the center of the plot, which was then averaged.

4.2.2. MICROCLIMATE AND THERMOREGULATION MEASUREMENTS

While the overall climate in an ecosystem can be monitored on a broader scale using meteorological stations, microclimate monitoring can range on a much smaller scale, including portable microsensors that can record atmospheric conditions at a smaller scope that would be experienced by an individual organism. Technology and data sensors vary in precision, where variation in method deployment further complicate comparison, such as placement of sensors within the environment, exposure to solar radiation, latitude, distance from the ground and canopy cover (Körner, 2007; Lundquist & Huggett, 2008; Monteith, 1972; Morecroft *et al.*, 1998). To assess the thermal properties of sleeping sites, I placed hygrochron iButton climate loggers (Maxim/Dallas Semiconductor Corp., USA) inside 12 confirmed sleeping sites of eight adult slow loris individuals (four males, four females). I placed loggers ~5 m below sleeping individuals, in attempt to avoid disrupting sleep behaviour. Maxim iButton loggers are the second most frequented climate sensors used to measure temperature and humidity in field ecology (Bramer *et al.*, 2018). These loggers are small in size, making them most feasible for placing in small potential microclimate area, particularly those with potential to be microrefugia. Loggers recorded measurements of ambient temperature at 5-minute intervals with an accuracy of $\pm$ 0.5 °C, and were used to measure internal temperature (T_i) of sleeping sites. In order to capture

the full range of a microclimate, sensors should remain in a single location, with two weeks representing a minimum duration to accurately measure the range of a site's conditions (Bramer *et al.*, 2018; Lembrechts *et al.*, 2018; Scheffers *et al.*, 2013). Therefore, once loggers were placed within a sleeping site, loggers remaining recording temperature and humidity for two weeks, to accurately measure any microclimate variation.

Following Schmid (1998), I simultaneously recorded corresponding external temperature (T_o) for each T_i, to distinguish any possible microrefuge. Climate loggers used to measure T_o were placed approximately 10-15 m due North of the sleeping site on the same geographic slope. Climate loggers collected measurements of ambient temperature (T_a) and percentage of relative humidity (rh%) at 5-minute intervals with an accuracy of ±0.5°C. I rotated climate loggers bi-weekly between individual sleeping sites from February until July 2016.

To determine thermoregulation of slow lorises in relation to their sleeping sites, I attached skin temperature loggers to the inside of individual radiotransmitter collars (section 2.3.1). I attached T_{sk} loggers to the radiocollars of the same slow lorises that had microclimates monitored for their sleeping sites, to determine if sleeping sites selected aid in behavioural thermoregulation.

4.2.3. STATISTICAL ANALYSES

GPS data can be analysed using various estimators, including minimum convex polygons (MCP), Kernel estimates, and harmonic mean (Larson & Rogers, 1997). These estimators each eliminate assumption of GPS point distribution and vary in strengths and weaknesses. When measuring home ranges using GPS data, researchers have found different software programmes to also yield different values (Larkin & Halkin, 1994; Lawson & Rogers, 1997).

While Kernel estimation is least biased allowing consistent comparison, MCP analyses are most consistent across software programmes with least variance (Harris *et al.*, 1990). Furthermore, MCP can identify areas which have a high density of an animal's locations (Burt, 1943; Kenward, 1987; White & Garrott, 1990). As slow lorises have been observed to re-use sleeping sites (Nekaris *et al.*, 2017), and are expected to display a high density of sleeping sites in repeated locations. Therefore, to show sleeping site selection by slow lorises within their homeranges, I plotted sleeping sites used by four social groups using 95% MCP estimates in ArcGIS v10.4, to account for density of site locations. Each social group consisted of one adult female and one adult male, as they use the most stable home ranges in this population.

When animals slept in bamboo, I used the bamboo stand as the sleeping site unit of analysis, following Nekaris *et al.* (2017). I calculated the temperature difference within bamboo sleeping sites (T_i – T_o) to measure potential microclimate variation between selected bamboo and immediate outer ambient temperatures (Schmid 1989). All values less than 0.0±0.9°C represented the lack of a microclimate, whereas a positive value (e.g. ≥1.0°C) represented a warming insulation of bamboo and a negative value (e.g. ≤0°C) represented a cooling effect. I tested all temperature data for auto-correlation using a Spearman's rank correlation test, and then used a Wilcoxon signed-rank test to compare T_i and T_o. I present all mean values ± Standard Deviation in the Results section.

To determine behavioural thermoregulation, I exclusively analysed T_{sk} data from individuals that simultaneously had a climate logger within their sleeping site. To account for T_a influence on T_{sk}, I only used data during daytime resting behaviour where the animal is immobile in its sleeping posture, to account for possible T_a influence on T_{sk} measurements

(Dausmann *et al.* 2004; see section 3.2.3). Simultaneous behavioural observations (sections 2.3.1, 2.3.2) were used to determine when individuals are inactive and in their typical sleep posture. As active locomotion can cause external loggers to become exposed to ambient temperatures (possibly skewing T_{sk} recordings), I only use T_{sk} measurements during behavioural inactivity to identify torpor bouts (Dausmann, 2005; Dausmann *et al.*, 2004; Kobbe & Dausmann, 2009; Nowack *et al.*, 2010).

For all climatic and model analyses (see Table 2.5), I grouped data in hourly fixed-effect time blocks to determine if time correlated to the response variable (T_{sk}) across a 24-h period (Litell *et al.*, 2000). Time blocks avoid pseudo-replication of samples in using time as a coefficient in the model, making time a categorical variable. Additionally, time block intervals are ideal for determining microclimate responses, as microclimates may only deviate from the atmospheric temperatures temporarily throughout a 24-h period (Bramer *et al.*, 2018). I excluded torpor bouts as outliers, because *Nycticebus javanicus* is a heterothermic primate (see Chapter 3) and torpor use will display a physiological mechanism of lowering of T_{sk}, and will not tell us about the influence of sleeping site characteristics on behavioural thermoregulation. After omitting all inconsistencies, I ran linear models using the lme4 package (Bates *et al.* 2015) and MuMIn (Barton 2016) in RStudio (R Core Team 2016).

As my research question was to determine what influenced the T_{sk} of *N. javanicus* during daytime resting behaviour in sleeping sites, I used linear models to test this hypothesis. T_{sk} is stored at continuous intervals over multiple days, where time variables are repeated but data wer not consistently taken on behaviour and location for each T_{sk} data-point. Models allow for flexibility of covariate measurement types. Thus, I fitted these data to a linear

model, as the response variable I tested for (T_{sk}) is a continuous variable and the independent variables fitted to the model range are categorical.

Models are analyses best suited for animal observation data that has repeated measurements (of the same unit) over time and space (Goonewardene *et al.*, 2000; Wang & Goonewardene, 2004). Repeated measurements on a single individual are more likely to be correlated to one another, and even more so for repeated measurements taken at closer time intervals, resulting in auto-correlation of data measurements, a problem commonly face in the use of telemetry data (Azzalini, 1994; Rooney *et al.*, 1998). Mixed models account for these correlations through allocation of a randomised variable, such as the individual (if the experiment is conducted across a population) or a randomized covariate measurement within an experiment of a single animal (Littell *et al.*, 1998). These are common analyses used in animal physiology, to understand weight change or metabolism over time during an experiment, where repeated measurements are allocated as fixed-effect blocks (Liang *et al.*, 2017; Silvia *et al.*, 1995; Goonewardene *et al.*, 2000; Nogueira *et al.*, 2019; Sucupira *et al.*, 2019). Furthermore, mixed models allow for gaps and inconsistencies in the collective data measurements over time (Templeman *et al.*, 2002).

I analysed use-availability surveys via logistic regressions to determine the different vegetation species used by *N. javanicus*. As I was testing hypotheses on bamboo sleeping sites, I grouped all surveyed vegetation species as 'bamboo' and 'non-bamboo', using a t-test statistical analysis. I used descriptive statistics to calculate the percentage at which *N. javanicus* used different sleeping site types. While compositional habitat analyses can be used to provide a robust understanding of habitat availability and selection by free-ranging animals (Bingham et al., 2007), these analyses would provide an interpretation of habitat

used by slow lorises across all activity patterns, and thus were not necessary for the assessment of sleeping site selection and desnsity. Furthermore, CA provides larger error-rates when more animals are included in the model with equal observation ratios (Bingham et al., 2007), providing less reliable for a long-term dataset with many focal animals such as LFP. To test what sleeping place *N. javanicus* most frequently slept in, I divided the tree height into three vertical portions (lower, middle, upper) and compared the sleeping place height using a Chi-square test.

4.3 RESULTS

4.3.1. HABITAT USE AND SLEEPING SITE SELECTION

I found *N. javanicus* to use ≥50% of the botanical species recorded in Cipaganti, although bamboo (*Gigantochloa atter*) and eucalyptus (*Eucalyptus* spp.) were the most available taxa (Table 4.1). During habitat surveys, 89.58% of the habitat used as sleeping sites by *N. javanicus* were in bamboo (*G. atter* (n=40), *G. pseudoarundinacea* (n=3)), while the remaining available vegetation were non-bamboo species, collectively representing ~10% of all sleeping sites (Table 4.1). The average canopy cover of bamboo (91.96 ± 8.55%) was significantly higher than non-bamboo vegetation (50.63 ± 33.5%; t_{658}=12.765, P< 0.001). Bamboo displayed a lower DBH (30.30 ± 12.25 cm; t_{364}= -9.912, P < 0.001) compared to non-bamboo species surveyed (DBH: 66.06 ± 37.76 cm), with the exception of coffee plants. Tree heights of bamboo and non-bamboo species were virtually identical (bamboo, 9.39 ± 2.57 m; non-bamboo, 9.64 ± 3.14 m.

Table 4.1. Surveyed habitat in Cipaganti, measuring used and available plant species from random plot surveys. We defined botanical species as 'used' by the presence of *Nycticebus javanicus* in the vegetation type, and unused when *N. javanicus* was absent. We used the equation: (no. available sites + no. used sites) = n value for Resource Selection Functions, as per Johnson *et al.*, 2006. We consider both used and unused vegetation recorded in plots to determine average tree height, tree height range, average diameter at breast height (DBH) and canopy cover (mean ± SD).

Family	Plant species	no. available sites	no. used	RSF no.	% used of available	no. used as sleeping sites	% of sleeping sites	Vegetation height	Height Range	DBH	Canopy Cover
Poaceae	*Gigantochloa atter*	105	84	189	80	40	**83.33**	9.30 ± 2.45	3--13	29.52 ± 11.62	91.69 ± 8.48
Poaceae	*G. pseudoarundinacea*	10	6	16	74	3	6.25	10.40 ± 3.63	6--14	38.5 ± 16.51	91.677 ± 9.89
Lauraceae	*Persea americana*	23	17	40	73.9	0	0	9.49 ± 3.07	2--17	56.92 ± 38.09	55.75 ± 34.11
Meliaceae	*Toona sinensis*	18	15	33	83.33	0	0	8.67 ± 1.71	6--12	73.12 ± 20.58	83.77 ± 7.53
Fabaceae	*Acacia decurrens*	35	25	60	71.4	0	0	9.17 ± 1.79	6--12	59.51 ± 20.63	78.97 ± 19.68
Fabaceae	*Calliandra spp.*	20	13	33	65	0	0	6.75 ± 2.05	4--10	44 ± 30.27	91.27 ± 4.08
Myrtaceae	*Eucalyptus spp.*	103	77	180	74.76	2	4.17	9.0 ± 0.7	5--17	66.11 ± 24.51	75.41 ± 12.87
Moraceae	*Artocarpus heterophyllus*	3	3	6	100	1	2.08	11.7 ± 4.73	8--17	72.33 ± 110.9	93.37 ± 4.6
Rhamnaceae	*Maesopsis eminii*	11	8	19	72.73	0	0	9.5 ± 2.99	2--17	55.21 ± 36.34	58.09 ± 34.11
Ebenaceae	*Diospyros kaki*	6	5	11	83.33	0	0	7.0 ± 1.0	6--8	74.4 ± 21.61	81.28
Cassuarinaceae	Casuarina junghuhniana	15	10	20	66.67	0	0	10.93 ± 1.39	9--13	60.6 ± 30.20	83.68 ± 3.57
Rubuaceae	*Coffea arabica*	4	4	8	100	0	0	2.25 ± 0.29	2--2.5	6.0 ± 2.94	86.03 ± 13.02
Cucurbitaceae	*Sechium edule*	7	7	14	100	0	0	2	2--2	n/a	n/a
Magnoliaceae	Manglietia blumei	1	1	2	100	1	2.08	10	n/a	86	n/a
Solanaceae	*Cestrum aurantiacum*	1	1	2	100	1	2.08	5	n/a	10	n/a
Proteaceae	*Grevillea robusta*	4	2	6	50	0	0	13.0 ± 2.31	11--15	84.0 ± 6.93	77.64 ± 0

The slow lorises most frequently used bamboo stands as sleeping sites, showing no pattern of distribution within home ranges (Figure 4.3). When the sleeping place could be detected (n=521), slow lorises slept in the upper third portion of sleeping sites (X^2=148.55, df=26, p=<0.0005), with females (n=320) choosing the upper third portion more than males (n=118) (X^2=12,226, df=2, p=0.002). The slow lorises slept on a single culm towards the center of a bamboo stand, or wedged between a culm and a branch.

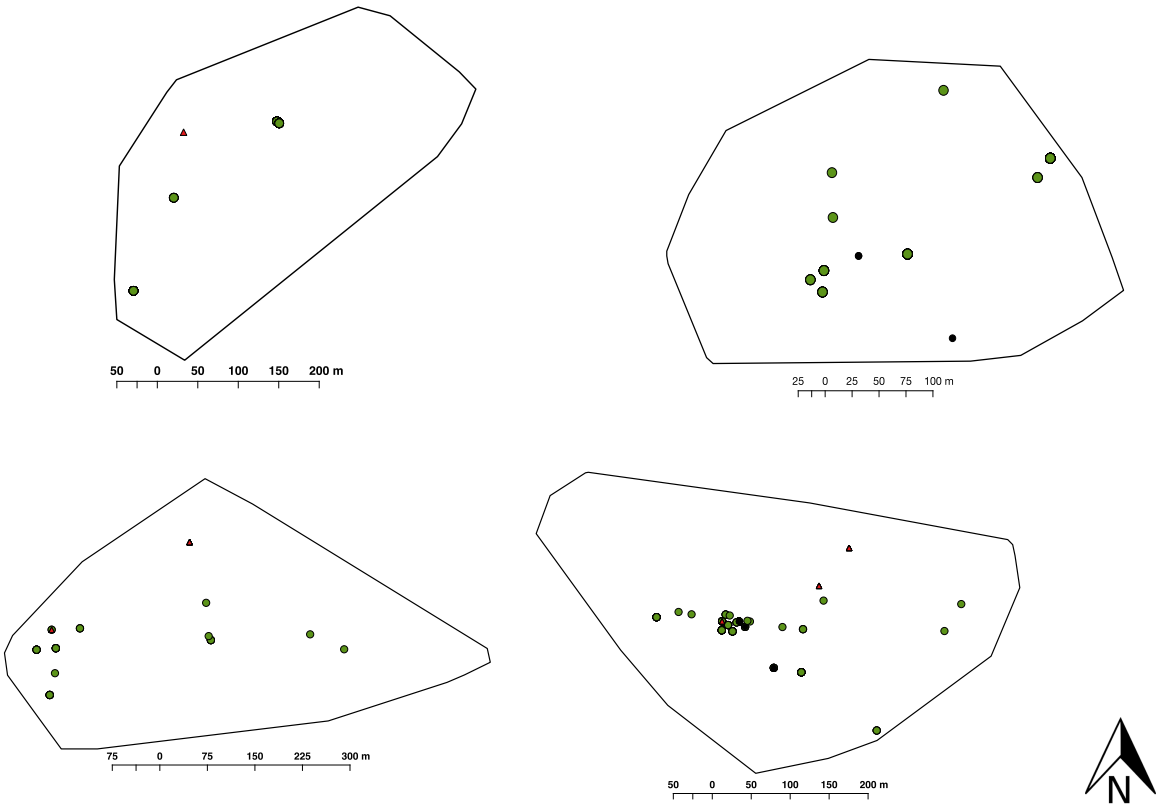


Figure 4.3. Preferred sleeping sites of *Nycticebus javanicus* social groups at Cipaganti between 2016 and 2017. Solid lines represent the borders of each group's home range. Green circles indicate bamboo sleeping sites, while red triangles indicate non-bamboo sleeping sites. Top left: Group 1 (adult male DP, adult female LU) ranging at 1289-1401 m asl. Top right: Group 2 (adult male AZ, adult female OE) ranging at 1418-1535 m asl. Bottom left: group 3 (adult male FE, adult female MA) ranging at 1376-1509 m asl. Bottom right: group 4 (adult male AL, adult female TE) ranging at 1346-1502 m asl.

I sighted small carnivores 1081 times (*Paradoxurus hermaphroditus* n=807; *Melogale orientalis* n=157; *Prionailurus bengalensis* n=70; *Herpestes javanicus* n=14*; Mydaus javanensis* n=7; *Martes flavigula* n=12; *Prionodon linsang* n=9; *Viverricula indica* n=3; *Panthera pardus* n=2). Only *P. hermaphroditus* was detected off the ground, with a minimum height of 0.1 m and a maximum of 12 m (average 3.3 $\pm$ 3.4). Civets used the following height classes: ground (25%); 0.1-4.9 m (39%); 5-.9.9 m (16%) and above 10 m (10%). All substrate use above 10 m occurred on water hose pipes that arboreal mammals in the area use as habitat connectors. Of the observed civets in bamboo, 185 were 1 m or below, with only 4 sightings at 2-3 m, and 4 sightings at 4-6 m.

Comparing all GIS referenced animal sightings in the area from 2014 until 2017, I found a total count of 72 leopard cats (*Prionailurus bengalensis*) and 161 civets (common palm civet, *Paradoxurus hermaphroditus*; small indian civet, *Viverricula indica*) sighted in total, with 47 leopard cats and 114 civets ranging within 0-25 meters of a slow loris sleeping site (Figure 4.4). Slow lorises were observed to wake within a bamboo stand when birds of prey flew over sleeping sites, and were observed to change sleeping place within a sleeping site when disturbed by small birds, within ~5 meters in the same bamboo stand.

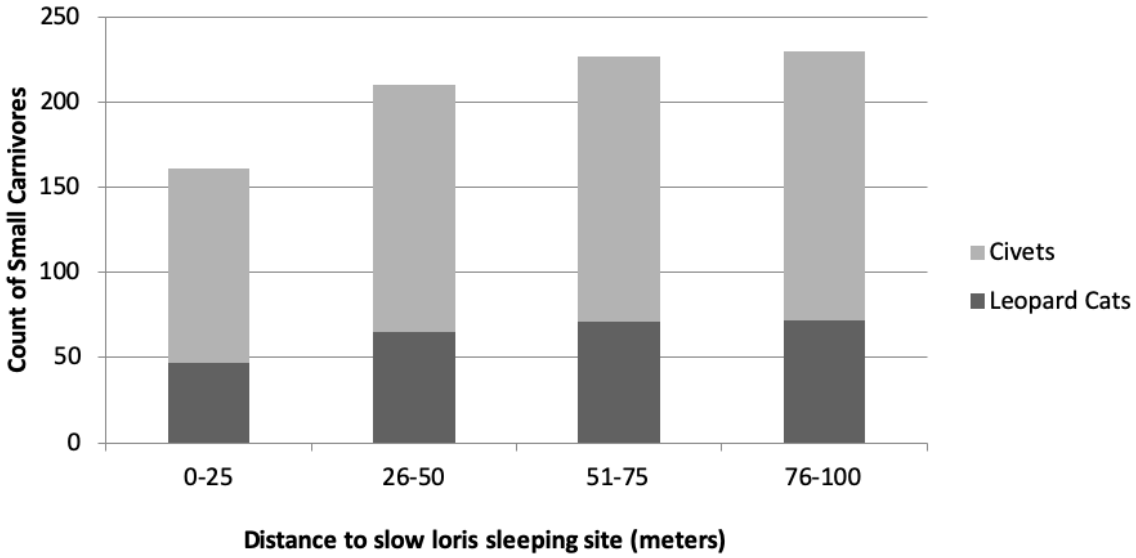


Figure 4.4. Proximity of small carnivores (civets and leopard cats) to Javan slow loris sleeping sites within the same years, from 2014 to 2017.

4.3.2. MICROCLIMATE VARIATION

Temperatures recorded inside bamboo sleeping sites (T_i) displayed a large positive correlation with T_o ($r(12818) = 0.7$, $P < 0.001$) where the linear relationship displayed an inverse relationship with humidity, and more outliers where humidity was lower (Figure 4.5). Nevertheless, bamboo sleeping sites displayed a buffered microclimate compared to ambient temperatures (Kolmogorov-Smirnoff, D= 0.23, $P< 0.002$), displaying a mean temperature of 21.2 ± 2.02°C, while maintaining a microclimate that is on average 0.9 ± 0.4°C warmer than sleeping sites in alternative species (20.3 ± 2.1°C*; Z*= -6.61, $P = 0.007$). Bamboo stands displayed a warmer microclimate variation, with a T_i range of 15.6 to 35.6°C, compared to the outer ambient temperatures of T_e ranging between 15.4 and 26.0 °C.

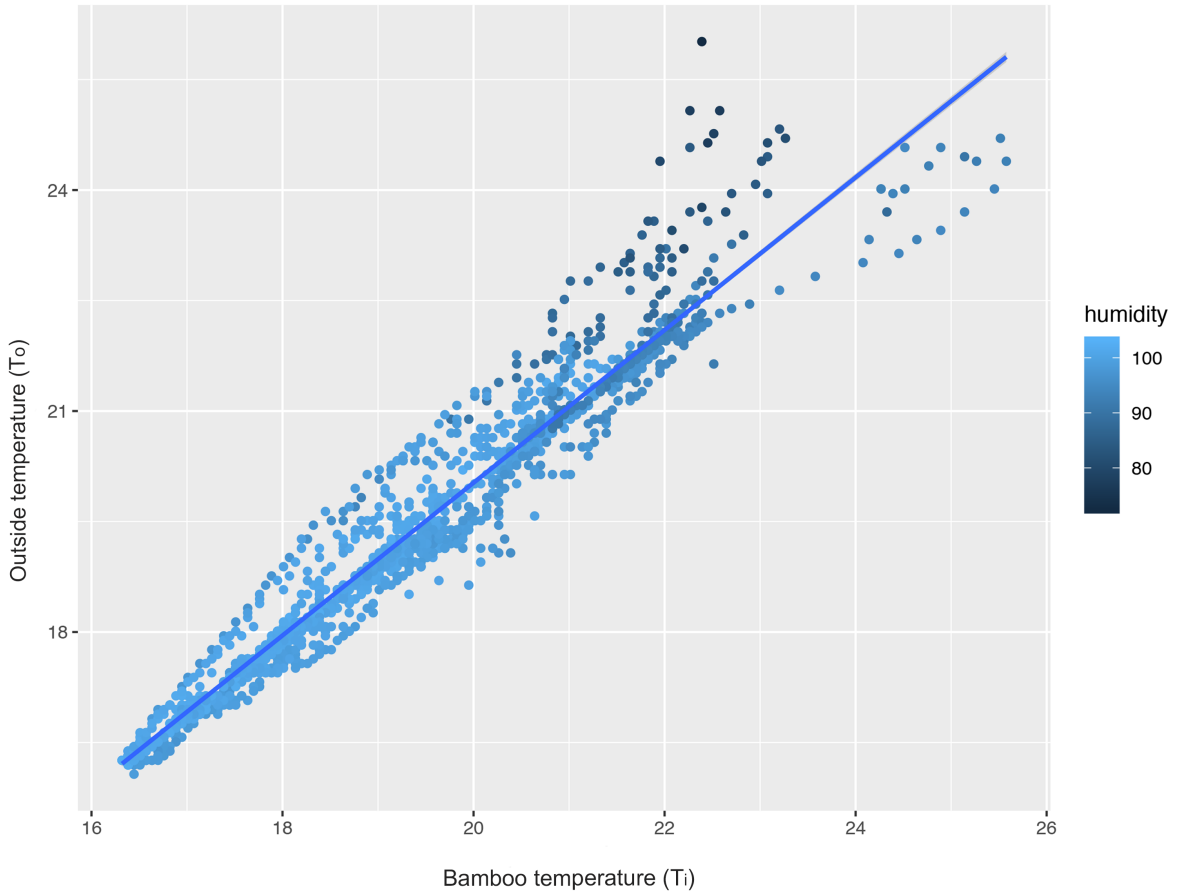


Figure 4.5. Linear relationship between bamboo sleeping sites microclimate (T_i) and outside temperatures (T_o), in relationship to outside relative humidity. Each variable is coded to its correlated humidity, where lighter blue represents higher relative humidity.

Bamboo stands displayed a buffering effect to outer T_a fluctuations, where T_i displayed a smaller range of temperatures during the hottest parts of the day while slow lorises slept, which maintained a more stable microclimate (Figure 4.6). When mean T_a dropped below 20°C (0:00 – 06:00) with respect to the previous night, mean temperatures within bamboo sleeping sites displayed positive insulation temperatures. Bamboo also displayed insulating capacities during slow loris active hours (17:00-05:00). Bamboo stands also had a significantly lower relative humidity with a mean 94.9±7.5 rh%, compared to outer relative humidity of 96.4±7.5 rh% (Z= -48.27, P< 0.001).

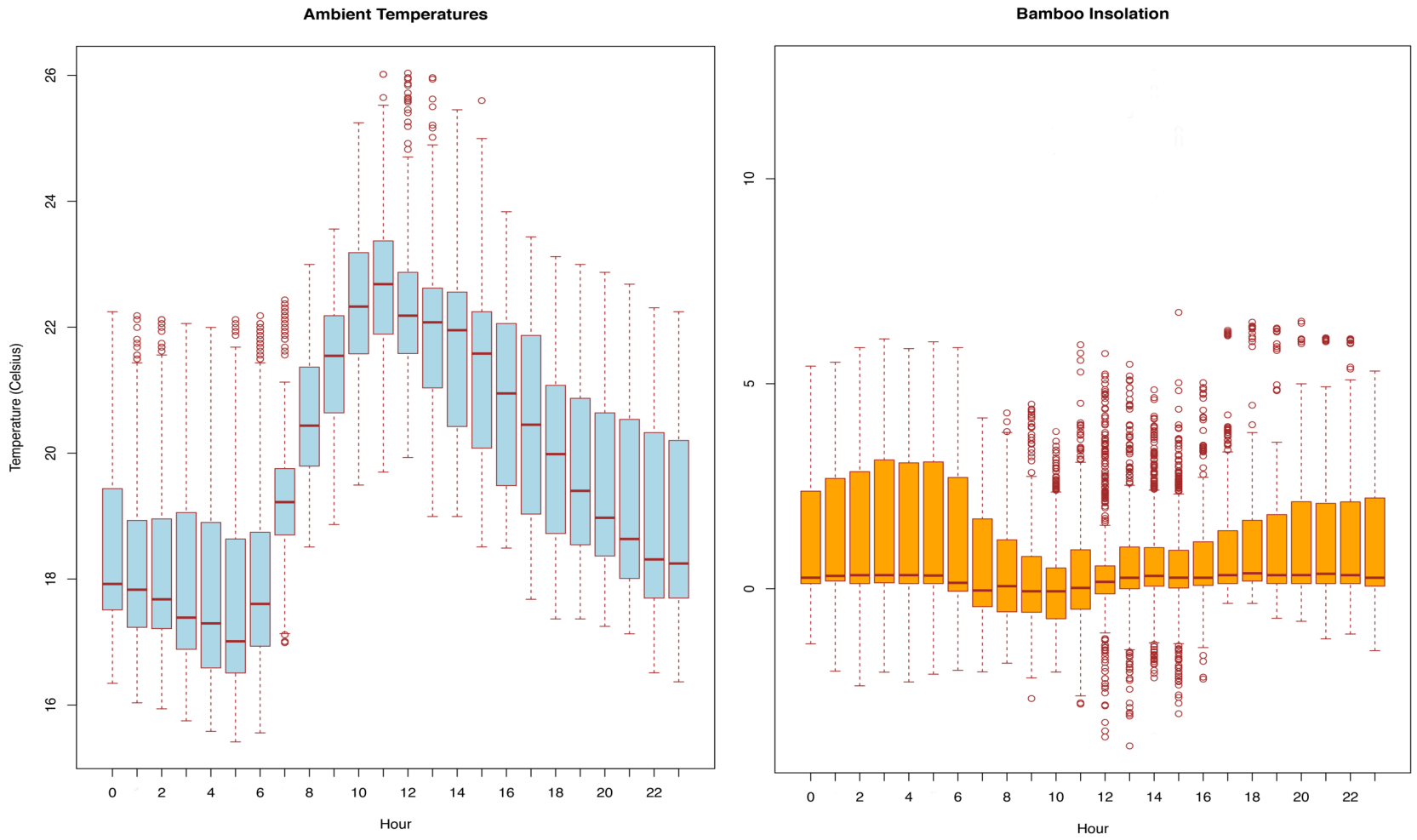


Figure 4.6. Left: Box plots of average climate variation of ambient temperature (T_o) within the home ranges of slow lorises (n=8) across a 24-h period. Open dots represent outliers. Right: Box plots of calculated insulation (T_i – T_o) of identified bamboo sleeping sites used by slow lorises across a 24-h period. Positive values represent a warmer insulation microclimate; negative values represent a cooling 'buffer' microclimate. Open dots represent outliers.

The regression indicated that the predictors (time of day, month, insulation capacity of sleeping sites and individual slow lorises) explained 30% of the microclimate variance within bamboo sleeping sites (R^2= 0.30, F (4, 1528)= 3.86, *P*< 0.001). Monthly variation had the lowest relationship, but still predicted T_i tendencies (Table 4.2).

Table 4.2. Linear model results, showing the influence of factors (time, month, insulation and individual) in predicting microclimate of bamboo sleeping sites.

Variables	ß	SE	*t*	*P*
Intercept	22.306	0.064	348.80	
Hour ***	0.063	0.002	26.096	<0.001
Month*	-0.104	0.047	-2.445	0.015
Insulation ***	-0.041	0.001	-39.76	<0.001
Individual***	1.684	0.107	15.711	<0.001

4.3.3. BEHAVIOURAL THERMOREGULATION

Of the slow loris individuals that simultaneously had T_{sk} loggers adhered to radio-collars, only six were successfully retrieved. Individuals displayed differences in their experienced microclimate in relationship to their selected sleeping sites. One Individual (Tombol: coded as '50') selected sleeping sites that were higher in T_i compared to other slow lorises, consistently throughout the 24-h period (Figure 4.7).

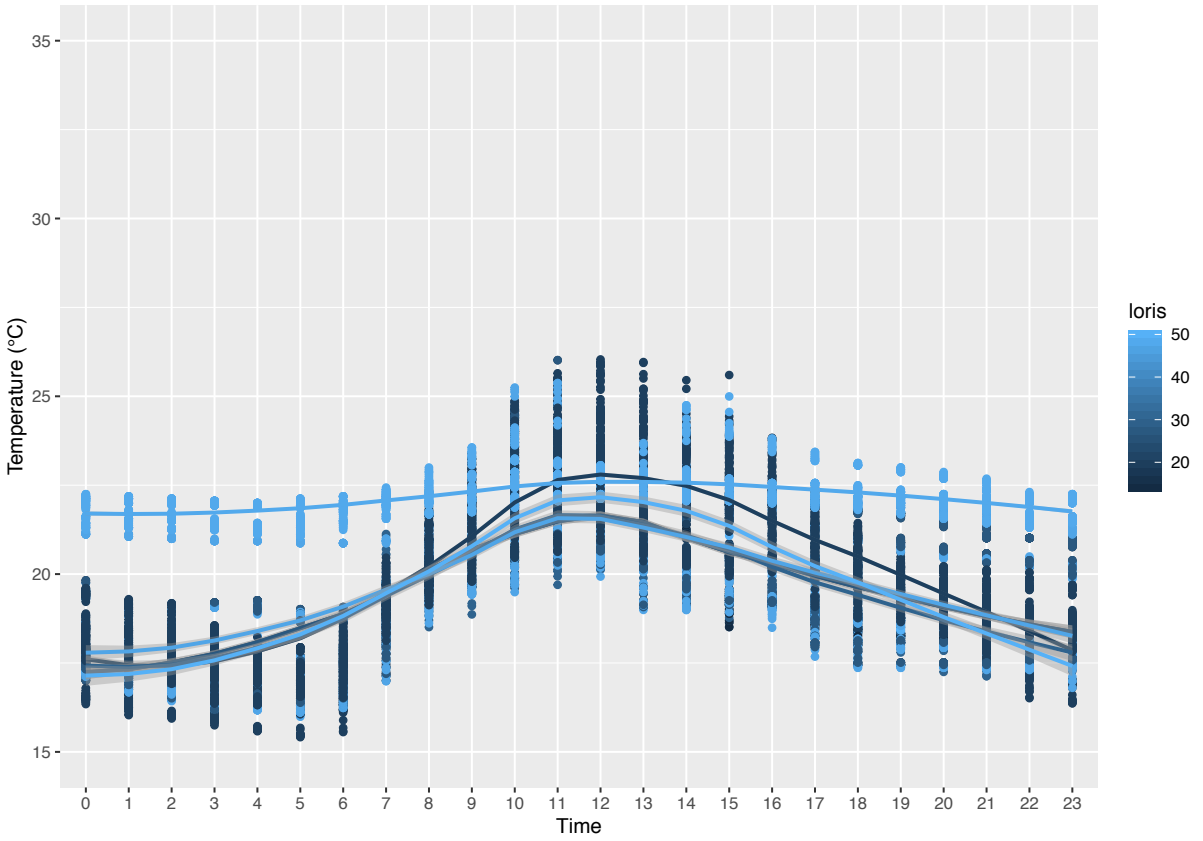


Figure 4.7. Microclimate temperatures (°C) experienced by individual slow lorises across their selected sleeping sites (*n*= 6). Individuals were coded in a gradient from light to dark blue, where dark blue represented adults and light blue represented juveniles.

Testing the selection of sleeping sites in relation to weather variables, rain, tree height and age were all found to influence the selection of bamboo as a sleeping site (Table 4.3). Ages found to be significant in the model predicting bamboo selection included infants and adults, while juveniles were largely insignificant. When comparing skin temperature of individuals throughout the duration of which they were inside their sleeping sites, slow lorises displayed relatively stable T_{sk}, ranging between a mean of 31 - 35.5 °C (Figure 4.8).

TABLE 4.3. Binomial logistic regression results in predicting the selection of bamboo as a sleeping site by Javan slow lorises. 'Age (1)' adult age category, 'Age (2) juvenile age category, 'Age (3)' infant category.

Variables	B	SE	Wald	df	*P*	Exp(B)
Rain *	0.547	0.218	6.31	1	0.012	1.729
Wind	0.048	125	151	1	0.698	1.05
Tree height (m)	-0.018	0.026	0.51	1	0.475	0.982
Age (1) *	1.11	0.495	5.02	1	0.025	3.034
Age (2)	774	0.567	1.862	1	0.172	2.169
Age (3) *	1.336	0.662	4.075	1	0.044	3.804
Constant	-1.62	0.552	8.622	1	3.000	0.198

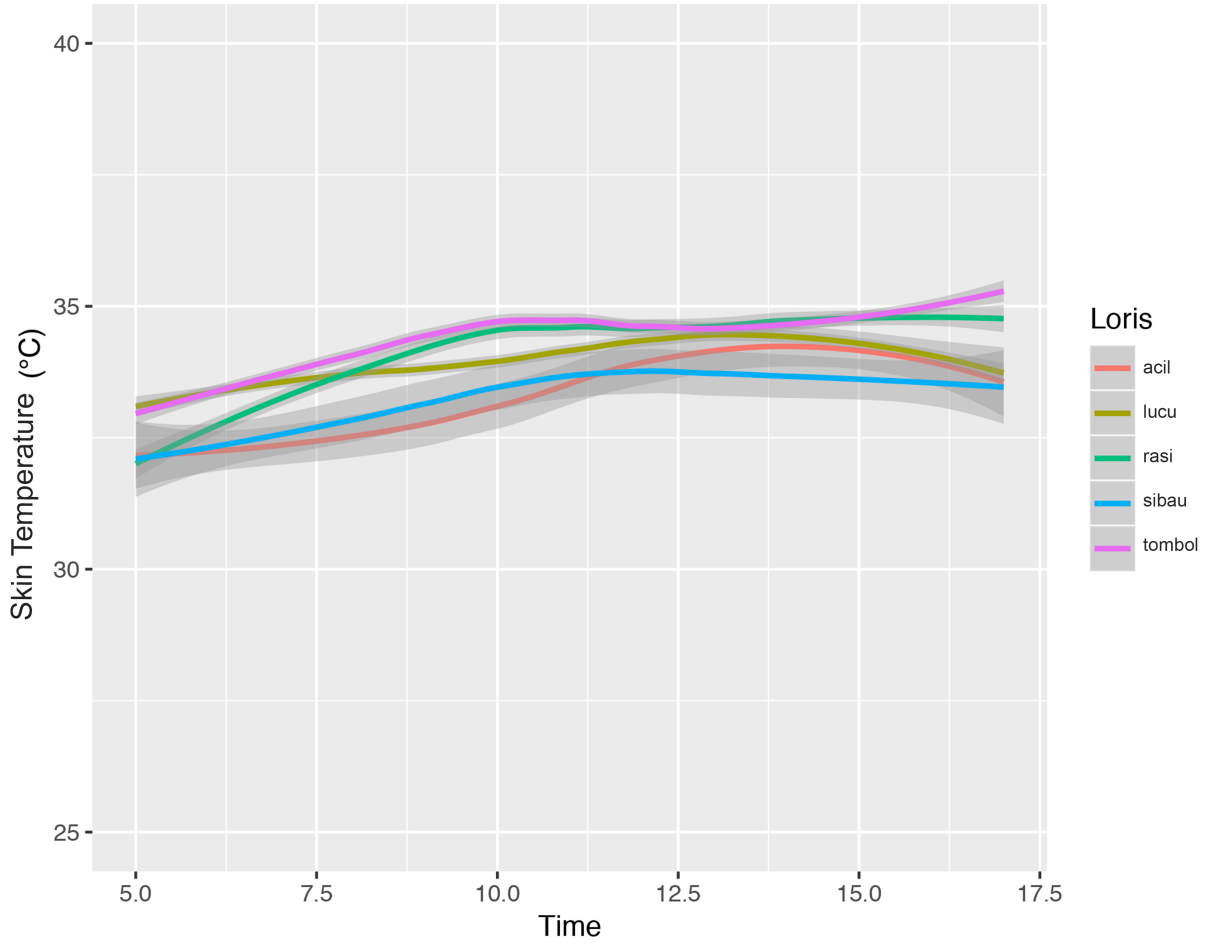


Figure 4.8. Skin temperature (T_{sk}) of slow lorises in bamboo sleeping sites during their resting hours, between 05:00 and 17:30 (x-axis). Each colour line represents a slow loris individual. Grey shading represents a loess line with 95% confidence. The y-axis scale represents skin temperature, across the range of recorded ambient temperatures.

4.4 DISCUSSION

Arboreal mammals often choose sleeping sites that are suitable for quick detection of or function as shelter from approaching predators (Anderson 1998; Caine 1987; Gautier-Hion 1970). Although there are many other vegetation types available in our study area, *N. javanicus* almost exclusively selected bamboo stands as sleeping sites. Bamboo stands provided more escape routes and a denser canopy cover than other vegetation types used by *N. javanicus*. Bamboo grows at a rate of about 1.6 inches per day, making it the fastest growing woody plant (Lobovikov 2007), and has been reported to grow in condensed clumps comprised of up to 100 culms in this region (Nekaris *et al.* 2017). Due to these constantly growing substrates, leaves and sheaths, bamboo provides an annual dense canopy cover (Lobovikov 2007). Despite sleeping in the upper third portion of sleeping sites, the dense canopy cover of bamboo may aid in decreasing detectability by birds of prey.

Bamboo stands are comprised of a dense arrangement and tangle of culms, that may enhance predator detection and vigilance. When an animal begins locomotion within a bamboo stand, individual culms will have a ricochet effect due to high density, arrangement and flexibility of substrates. The ricochet effect creates vibration, noise and possibly shedding of mature bamboo sheaths that could alert sleeping individuals (Barrazza 2012; Uetz 1989). Due to their cryptic behaviour and locomotion, slow lorises are virtually silent when entering bamboo, and remain silent throughout daytime rest. This cryptic locomotion combined with the frequent selection of bamboo as a sleeping site may decrease detectability by predators, while still allowing slow lorises to detect and avoid approaching predators.

While three species of nocturnal carnivore have been found within 0-25 m of slow loris sleeping sites, there have been no observations of these species predating on this specific population of *N. javanicus* by researchers or locals. Furthermore, in a review of predation by a felid nocturnal primate (Burnham *et al.* 2013), there was only one case recorded (out of 1,939 publications). While there are no reports of direct predation risks to slow lorises at Cipaganti, lack of evidence for predation does not dismiss predation as a selective pressure in sleeping site selection, and is likely to be an ultimate evolutionary pressure (Coleman & Hill, 2014). Furthermore, human presence alone has been observed to decrease predation by wild animals (Isbell 1994), and this is an area where there is virtually always human presence, as an agricultural landscape with farmers present during the day and field researchers present at night.

Risks of predation can have a strong influence on the spatial and microhabitat use of prey organisms, ranging from aquatic insects to lizards and desert rodents (Bramer *et al.* 2018; Fraser & Cerri 1982; Holomuzki 1986; Kotler 1984; Sih 1982, Stamps 1983). Small carnivores were most often sighted occupying the lower third of bamboo, (with civets never exceeding 6 m in spatial use), while *N. javanicus* most often occupied the top third vertical portion of sleeping sites. This niche difference in bamboo microhabitat use could be influenced by body mass and feasibility, aiding in predator avoidance. Use of bamboo for sleeping sites could also be due to anatomical comfort, in supporting sleep in typical sleep postures (Lima *et al.* 2005; Samson & Nunn 2012). All bamboo was recorded to have a significantly smaller DBH, which may be relevant for slow loris morphology and grasping abilities. Slow lorises have a unique morphological trait called the *retia mirabile*—a network of capillaries in the hands and feet. This capillary network is associated with a pincer-like grip formed by extreme reduction of the second digit. Both of these traits combined allow

slow lorises to cling onto a substrate while being immobile for extended periods of time, without going numb (Ankel-Simons 2000). This unique morphology aids to secure a grasp to swaying branches during strong winds, or defending against attacks and/or branch shaking from predators while demanding less energy exertion (Müller 1985; Nekaris 2014; Nekaris and Stevens 2007; Starr and Nekaris 2013). Such grasping abilities could form part of an anti-predator crypsis complex (Charles-Dominique 1977; Oates 1984; Nekaris 2014). Similarly, neuroscientists have suggested an additional antipredator strategy for sleeping animals called the 'Immobilization Hypothesis', which suggests animals in non-immediate predation risk are safer to remain quietly in deep sleep, as to not respond to non-threatening prompts, attracting predators to their presence (Meddis 1957, 1977). However, contrasting hypotheses, such as the 'Blackout Sleep Hypothesis', suggests that an animal is even more safe by remaining quietly awake (Lime *et al.* 2005). I suggest that the *retia mirabile* may have evolved as an antipredator strategy for both enhancing cryptic locomotion as well as prolonged immobile clinging for quiet, undisturbed sleep.

Organisms are known to select microhabitats for their thermal gradients, as well as food densities (Mittelbach, 1981). Primates of various body size have been observed to choose sleeping sites with heat retaining characteristics. Large primates such as *Pan troglodytes* may select sleeping sites that absorbed heat from sunlight during the day (Andersen 1982, 1998; Goodall, 1962; MacKinnon, 1974). Bamboo sleeping sites used by *N. javanicus* maintained a warmer microclimate, presumably from retained heat from the sun, compared to daily ambient temperatures. In addition to the regular warmer microclimate of bamboo, presence of heat retention from sunlight may be energy efficient for *N. javanicus* during waking hours (between 17:00 and 18:00), as mammals display limited thermoregulatory capacity during active sleep, and so heat retention can aid the initial

arousal from this state (Elgar, 1988). Bamboo insulation may likely be a result of the many unique properties of bamboo structure, in that each individual culm maintains an inner core temperature while providing overhead shade (Campanello *et al.*, 2007). By maintaining a more constant temperature, bamboo stands act as a stabilizing buffer, providing shelter and thermoregulatory benefits against random shifts in T_a.

If humidity levels increase while ambient temperatures are already above a species' thermoneutral zone, sleep quality can be significantly hindered (Manzar *et al.*, 2012). This is because evaporation of heat is restricted, inhibiting an animal's thermoregulation capacities, especially during sleep (Edgar, 2000; Havenith, 1999). While bamboo sleeping sites maintained a consistent insulation of ambient temperatures, they also displayed significantly lower relative humidity which may be even more important in the promotion of sleep during daytime rest (Fanger, 1970; Manzar *et al.*, 2012).

Kappeler (1998) suggested that behavioural and physiological thermoregulation can either replace or amplify the thermoregulatory benefits of shelter use (such as tree holes or nests) by strepsirrhine primates. The buffer found in bamboo sleeping sites is similar to tree holes and burrows chosen by non-flying mammals (Goldingay, 2011; Schmid, 1989). These resting site types are mostly used as an antipredator strategy, breeding sites and shelter by heterothermic species (Kappeler 1998). Heterothermy generally makes an individual inactive for extended periods of time, making them vulnerable to predation (Nowack *et al.*, 2013; Geiser, 2004; Eppley *et al.*, 2017). *Nycticebus javanicus* is a heterothermic primate, with the physiological capacity to lower their body temperature (see Chapter 3). Stimulus that can evoke torpid heterothermic animals into arousal include peaks and drops in T_a, anthropogenic noises and approaching predators (Harrison, 1965), though sensitivity

towards different stimulus varies from species to species (Lyman *et al.*, 1982). Thus, small mammals have been observed to most often choose nests, tree holes and burrows as both sleeping and resting sites, to protect them from predators and harsh environments that might evoke arousal from sleep (Bright & Morris 1991; Buck *et al.* 1991; Heldmaier *et al.* 1981; Masing *et al.* 2007; Mueller 1999). Similar to tree holes, bamboo may help amplify physiological thermoregulation, buffering stimulus that might evoke arousal from torpid bouts, and aid in passive rewarming post torpor bouts. While the model in predicting the selection of bamboo sleeping sites for its microclimate was weak, this could be due to the individual who displayed outlier behaviour in selecting sleeping sites that were consistently warmer (see Figure 4.7). Interestingly, this individual is a juvenile, which would also explain lack of significance in the binomial regression, where juveniles were insignificant in predicting selection of bamboo sleeping sites as shelter from weather variables. Furthermore, as shown in Chapter 3, this same individual (along with other juveniles) displayed deeper torpor bouts comparative to other individuals. Therefore, the selection of warmer sleeping sites may be necessary to passively aid torpor arousal for individuals with smaller body size.

Some primates deal with climatic variations by adjusting their home range to lower altitudes during the harsh, colder seasons (Cui *et al.* 2006, Liu & Zhao, 2004). In addition to sheltering from harsh climate conditions, some mammals must equally manage thermoregulation (Bishop, 1979; Cui *et al.*, 2006). Many species of slow loris, such as *N. javanicus*, are island endemic species with geographical restrictions. To cope with low T_a, primates will use strategies of behavioral thermoregulation, such as sun-basking or postural huddling to conserve body heat (Dausmann; Geiser, 2004; Kelley *et al.*, 2016). Slow lorises sleep in a very specific huddle posture called a sleeping ball, where they tuck

their head into their chest and sit perched for long periods of time (Nekaris, 2014). Huddling posture is used both solitarily (e.g. *Eulemur collaris*) and socially with conspecifics (e.g. *Galago moholi, Rhinopithecus bieti*), known as social huddling or social thermoregulation (Cui *et al.*, 2006; Donati *et al.*, 2011; Eppley *et al.*, 2017; Nowack *et al.*, 2013). As slow lorises sleep both alone or in contact with conspecifics, social huddling should also be of consideration in the influence of thermoregulation. It should be noted that we did not have any recordings of social huddling during the time of T_{sk} logger deployment during this study. Such positional behaviour and heat retaining sleeping sites aid in thermoregulation, decreasing the influence of sudden ambient temperature shifts, thus increasing daily energy saving. This makes slow lorises the only known heterothermic primates observed to not use tree holes (Bearder *et al.*, 2003; Ruf *et al.*, 2016), and supports the comparability of bamboo to tree holes, as a viable sleeping site type.

As an agricultural mosaic, farmers planted the majority of trees to enhance crop soils, distinguish property boundaries and decrease landslides and erosion on steep slopes. In this area, bamboo provides both livestock fodder and soil restoration for local farmers. Bamboo is thus of high economic and ecological wealth in West Java. In particular, rats were observed to experience population increases as a result of bamboo seed crops (Janzen, 1976). Bamboo also enriches and maintains moisture in surrounding soils, which provides more ground coverage for burrowing species, again, such as the bamboo rats (Wiles, 1981). Bamboo is an evergreen woody grass in the subfamily Bambusoideae (in the family Poaceae), of high economic and ecological importance. Due to its fast growth rate, bamboo is widely used by humans as a commodity for building houses, traditional instruments, paper pulp and even food (Dransfield & Widjaja, 1995; McClure, 1966). Certain agricultural practices allow a persistence of the overall plant in an environment,

such as limiting resource harvesting to mature bamboo, allowing younger plants and stems to continue growing (Nekaris *et al.*, 2017).

These data support the comparability of bamboo to tree holes, as a viable sleeping site for slow lorises. I conclude that bamboo is a multifunctional sleeping site, in its unique set of structural and thermal characteristics that aid in predator avoidance, shelter from weather and buffering ambient temperatures to help in thermoregulation during sleep and torpor expression.

In this chapter I have shown that *Nycticebus javanicus* most frequently selected bamboo stands for sleeping sites. The high density of culms within a bamboo stand provides a high level of escape routes and canopy cover, enhancing the benefits bamboo stands may provide against predation. Bamboo has a significantly smaller DBH compared to other available vegetation species, which may aid in stability and grasping in sleeping sites. Bamboo provided a buffer against temperature drops during the day (08:00 until 16:00) and retained heat during the alternate hours, maintaining a distinct microclimate both compared to outer T_a and non-bamboo sleeping sites (*Eucalyptus spp., Persea americana*). This data suggests that the selection of bamboo as sleeping sites is both part of *N. javanicus* thermal strategy in maintaining normothermia during daytime resting behaviour, when ambient temperatures peaked or diverged outside of the TNZ, while also promoting sleep behaviour as an antipredator strategy. In the next chapter, I discuss how sleep patterns and circadian rhythms are influenced by variations in ambient temperatures, sleeping site temperatures and light environment. I also discuss the regulation of sleep in the overall physiological maintenance of *N. javanicus*, similar to temperature regulation.

Figure 5.1. An adult male slow loris (Mo) fitted with an accelerometer (Actiwatch mini) to its the radiotransmitter collar. Photo by M Williams.

Birds and mammals can display two different sleep patterns: monophasic sleep, when an animal exhibits a single consolidated bout of sleep in one portion of a day; or polyphasic sleep, when an animal displays several short episodes of sleep (Ball, 1992; Lima *et al.*, 2005; McNamara *et al.*, 2010}. Even in relatively stable laboratory conditions, the amount and characteristics of sleep and waking vary substantially across 24-h. These variations are superimposed with daily fluctuations in environmental variables that have a strong influence on activity patterns. Such fluctuations include risk of predation, ambient temperature, humidity and light environment (Broughton, 1973; Lesku *et al.*, 2006; Lima *et al.*, 2005; Peirson *et al.*, 2018; Siegel, 2005). The effect of these extrinsic factors on sleep and activity is mediated by their interaction with endogenous regulatory mechanisms, such as the circadian clock. The circadian clock provides a rhythmic output to behaviour and physiology, and is synchronised to light levels, allowing animals to anticipate day and night (Jud *et al.*, 2010; Kavanau & Peters, 1976; Peirson *et al.*, 2018). Another important intrinsic factor, which has a strong influence on sleep amount and intensity, is preceding sleep-wake history.

Numerous laboratory studies suggest that sleep is homeostatically regulated. The intensity of sleep increases as a function of preceding wake duration, with lowest sleep pressure towards the end of a sleep period (Borbély & Achermann, 1999; Dash *et al.*, 2009; Huber *et al.*, 2004; Vyazovskiy *et al.*, 2007). An important manifestation of sleep homeostasis is the capacity to compensate for the loss of sleep following sleep (or rest) deprivation (Rechtschaffen, 1998; Tobler, 1985). An animal that experiences a stimulus that arouses them from sleep during regular circadian rest patterns would need to reallocate resting time at another portion of the day (Lima *et al.*, 2005). Sleep rebound is regularly observed in laboratory animals, and suggests importance in the restorative function of sleep, and the

detriments an animal can experience should amount of time for sleep be compromised (Rattenborg *et al.*, 2004; Rechtschaffen, 1998; Rechtschaffen & Bergmann, 2002; Siegel, 2003; Tobler, 1985; Tonini & Cirelli, 2003). No studies on forced sleep deprivation have been performed in the wild, where I surmise that compensation for sleep loss would need to be balanced against environmental pressures. One wild study conducted on African elephants *(Loxodonta africana)* found animals to display no evidence of sleep rebound in response to deprivation from previous sleep (Gravett *et al.*, 2017), although the environmental cause for deprivation was unknown.

Sleep patterns of only a few mammalian species have been systematically studied in their natural habitats, with essential information on sleep patterns in wild primates still lacking (Davimes *et al.*, 2018; Gravett *et al.*, 2017; McNamara *et al.*, 2008; Rattenborg *et al.*, 2008). This is largely due to the fact that polysomnography can be particularly difficult to conduct in the wild and has only been done on a few captive primate species (lemurs, monkeys, baboons and chimpanzees), leaving many gaps in our knowledge on the accurate details of sleep (Nunn *et al.*, 2010; Samson and Shumaker, 2015).

Mammals exhibit a wide range in the amount of time spent asleep, with humans devoting nearly a third of their life in a state of sleep (Zepelin, 2000). Sleep duration varies greatly between primate species, experiencing a range of 60-120 minutes in REM per sleep cycle (Table 5.1). Evidence suggests that nocturnal primates display on average longer sleep durations (13-17 hours daily) compared to diurnal species that sleep for 8-11 hours (McNamara *et al.*, 2018; Nunn *et al.*, 2010). Captive studies of nocturnal primates (African lesser bushbaby *Galago* spp., Sumatran slow loris *Nycticebus coucang*, Northern owl

monkey *Aotus* spp.) displayed strictly nocturnal activity [Kavanau & Peters, 1976], but it is unclear whether these patterns extend to the wild [Allison & Cicchetti, 1976; Anderson, 1998; Bednekoff & Lima, 1998; Elgar, 1989]. Conducting comparative sleep research in primates, researchers reconstructed our ancestral primate sleep traits, suggesting an average of 11 hours daily, with 10 hours in NREM sleep and only 1.3 hours in REM sleep (Nunn *et al.*, 2010).

Table 5.1. A list of all published studies to-date on primate sleep as of April 2019, describing the species of study, identified activity patterns, recording method, and sleep characteristics (total sleep time, REM/NREM duration.

Common name	Species	Activity patterns	Data collection method	TST	REM duration	NREM duration	Refs
Three-striped night monkey	*Aotus trivirgatus*	nocturnal	EEG	16.97	1.82	15.15	11
Common marmosets	*Callithrix jacchus*	diurnal	electroacoustic	9.5	1.61	7.9	13
Vervet monkey	*Chlorocebus aethiops*		EEG	9.77	0.65	9.044	4
Patas monkey	*Erythrocebus patas*	diurnal	EEG	10.9	0.86	9.99	
Crowned lemur	*Eulemur coronatus*	Cathemeral	Accelerometer	8.96			
Blue-eyed black lemur	*E. flavifrons*	Cathemeral	Accelerometer	8.84			
Black lemur	*E. macaco*	Cathemeral	In press	9.65	0.84	8.81	
Mongoose lemur	*E. mongoz*	Cathemeral	Behavior	11.9	0.72	11.16	
humans	*Homo sapiens*	diurnal		7	1.56	5.41	14
Ring-tailed lemurs	*Lemur catta*	Cathemeral		11.05			
Macaques	*Macaca arctoides*	diurnal		9	1.38	7.65	
Long-tailed macaque	*Macaca fascicularis*	diurnal		10.46	1.71	8.74	
Rhesus macque	*Macaca mulatta*	diurnal	EEG; videography	10.23	2.05	8.19	5
Southern pig-tailed macaque	*Macaca nemestrina*	diurnal		9.88	0.99	8.89	
Bonnet macaque	*Macaca radiata*	diurnal		9.1	1.05	8.06	
Barbary macaque	*Macaca sylvanus*	diurnal	EEG	11.74	1.07	10.68	
Dwarf lemur	*Microcebus murinus*	nocturnal		15.36	0.99	14.37	6
chimpanzee	*Pan troglodytes*	diurnal	EEG	9.67	1.45	8.22	3, 7
baboon	*Papio anubis*	diurnal	EEG	9.84	1.39	8.45	2
Hamadryas baboon	*Papio hamadryas*	diurnal	EEG	9.83	1.27	8.61	
Guinea baboon	*Papio papio*	diurnal	EEG	10.07	1.06	9.01	

potto	*Perodictucus potto*	nocturnal		11			
Fork-marked lemur	*Phaner furcifer*	11.5					
Orang-utan	*Pongo pygmaeus*	diurnal	videography	9.11	1.11	8	12
sifaka	*Propithecus coquereli*	diurnal		10.63			
	Saguinus oedipus	siurnal	accelerometer	13.18			9
Squirrel monkey	*Saimiri sciureus*	diurnal	EEG	9.72	1.77	7.8	1
Gelada monkey	*Theropithecus gelada*	diurnal		10.91			10
Red-ruffed lemur	*Varecia rubra*	cathemeral		9.81			
Black-and-white ruffed lemur	*Varecia variegata*	Cathemeral		10.9			

In this chapter, I aim to bridge this knowledge gap on the environmental drivers of sleep in wild primates by conducting the first study on sleep patterns in a wild nocturnal primate, the Javan slow loris *Nycticebus javanicus*. To do this, I measured behavioural sleep (rest) of *N. javanicus* in the wild using accelerometer devices as a proxy for sleep (Hoffmann *et al.*, 2012; Mann *et al.*, 2005). I tested the influence of environmental factors on the daily activity patterns and sleep behavior of *N. javanicus*, considering the following questions: i.) What behavioral sleep patterns did individuals display, particularly focusing on rest duration and the time of day entering and exiting behavioral sleep?; ii.) Did *N. javanicus* perform consolidated monophasic or polyphasic resting patterns?; iii.) Did light and/or temperature mediate their activity-rest patterns? iv.) Did *N. javanicus* display sleep patterns that might suggest homeostatic sleep regulation?

5.2. MATERIALS AND METHODS

I recorded locomotor activity of slow lorises (n=14; 8 males, 6 females) as a proxy to interpret activity levels of *N. javanicus* between June 2014 and April 2018 (1,530 collective days; Figure 5.2). For accelerometer attachment methods and animal ethics, see Section 2.3.1 ‘Capture and Collaring’.

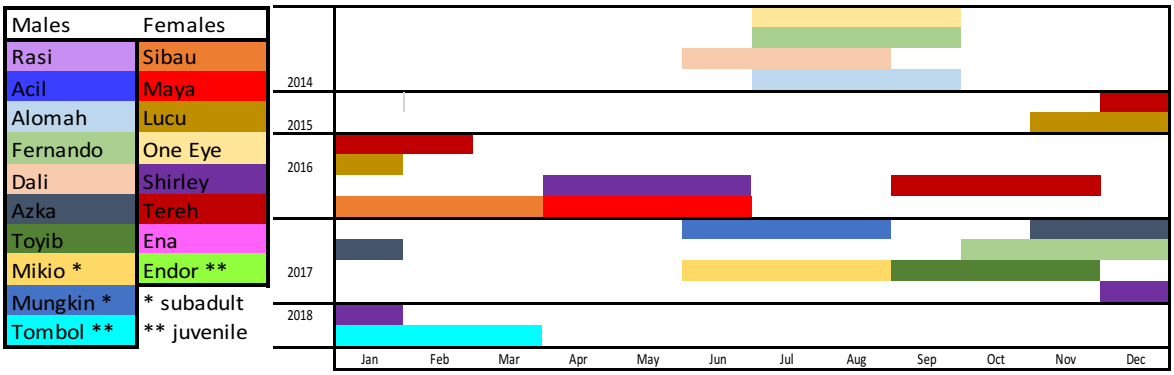


Figure 5.2. Diagram displaying timeline of accelerometer logger rotation between individuals. Each colour represents a unique individual.

5.2.2. MEASURING ENVIRONMENTAL FACTORS

To understand their entrainment and biological clock in response to their environment, I gathered data on the climate and light environment. Ambient temperatures were measured within the home ranges of focal animals, throughout the duration in which they had accelerometer recordings (section 2.3.4). I gathered all sunrise and sunset times, as light levels can be derived from these. I acquired sunrise and sunset times from an online world clock source (Time and Date AS 1995-2018) in the appropriate time zone (GMT +1) at the study site to test for circadian synchronization.

Visual responses to light environment can be studied through the monitoring of anatomical responses to light, such as measuring retinal function to the brain (electroretinography; Cameron *et al.*, 2008), photoreceptor density (Learney *et al.*, 2008), or pupillary responses (UV light sensitivity; Rohlich *et al.*, 1994; Jacobs *et al.*, 2004) requiring laboratory environments. Non-visual responses to light can be studied through measurements of melatonin suppression (Lucas *et al.*, 1999), activity onset/suppression or sleep (Pilorz *et al.*, 2016), and cognition (Tam *et al.*, 2016), often in conjunction with inbred laboratory mice with specific genetic mutations as study subjects. Alternatively, locomotor behaviour

has been vastly studied in both laboratory and field research to understand an organism's entrainment to their light environment (Peirson *et al.*, 2018).

5.2.3 .STATISTICAL ANALYSES

ACCELEROMETER VALIDATION

Accelerometer devices record movement and locomotion. One of the main defining characteristics of sleep is immobility, yet an animal may be awake but immobile (Resheff *et al.*, 2014; Sellers & Crompton, 2004). Specific behaviours can be extrapolated from activity scores using simultaneous behavioural observations, or using unsupervised algorithms for larger datasets or cryptic species (Brown *et al.*, 2013; Chimienti *et al.*, 2016; Sakamoto *et al.*, 2004). Most accelerometer models have a unique algorithm and technological method for measuring and storing data that represent movement and locomotion. Despite the efficiency of accelerometer use, the analysis of accelerometer data are not always simple and different methods have been employed, broadly classified as supervised and unsupervised learning algorithms (Sakamoto *et al.*, 2009; Brown *et al.*, 2013; Gris *et al.*, 2017). Validation should be done independently for each species, and ideally, for each animal wearing an accelerometer device (Halsey *et al.*, 2011).

Most primate studies using accelerometers used supervised learning algorithms to extrapolate behaviours from accelerometer data (Brown *et al.*, 2013). In the supervised learning algorithm, it is required to associate part of the accelerometer data manually with the corresponding behaviour to create the training data necessary for the algorithm to operate. For example, if you wanted to compare activity and inactivity using accelerometers, you could validate this with simultaneous behavioural observations. Similarly, if you wanted to measure sleep architecture, you could validate with

simultaneous electroencephalogram (EEG) recordings of brain activity. Validations do not require a constant simultaneous recording for the entire duration of the accelerometer's recording, but rather a sample size large enough to compare and test your variables.

For validating behaviours and accelerometers, you would want to have behavioural data containing each of the behavioural categories you wish to quantify. After this first stage, the algorithm (e.g. K-Nearest Neighbour algorithm; Bidder *et al.*, 2014) is run to match the training data with the rest of the dataset. In spite of the relative simplicity of this method assisted by the availability of a new user-friendly software (Brown *et al.*, 2013), this approach is limited by several issues. The first issue is that this approach requires an extended prior knowledge on the behaviours of the species, or large dataset to match behavioural data with accelerometer data. These conditions make this approach unsuitable for cryptic species for which behavioural observations are challenging (Chimienti *et al.*, 2016). Secondly, the identification of behaviours depends on thresholds that are species-specific (Gómez-Laich *et al.*, 2008). The training data and the algorithms used on a related species thus may not be applied to obtain reliable activity estimations on a different study species (Halsey *et al.*, 2011). Thirdly, the massive amount of data produced by accelerometers makes manual identification of behavioural patterns difficult (Resheff *et al.*, 2014). Finally, the same behaviour might be associated with different combinations of accelerometer data, thus each behaviour should be linked to all the possible accelerometer data to avoid misleading interpretations of the results (Gris *et al.*, 2017). Therefore, when using supervised algorithms, these limitations should be considered when interpreting data analyses and results and if possible, avoided for cryptic nocturnal primate species. Due to the limited applications of the supervised learning algorithms, especially in

cryptic animals, recently the use of unsupervised learning algorithms has been suggested as a method to classify accelerometer data (Brown *et al.*, 2013; Chimienti *et al.*, 2016).

While there are a number of programs that can be used to calculate different equations from accelerometers, a program may be developed from the data of one specific species/study, where it may not be the most suitable analysis for other species. I extracted raw data (activity scores) from accelerometer devices using the same software used for launching the loggers (MotionWare or PRO-Diary (CamNtech Ltd., Cambridge, UK; see Appendix: Key Resources Table). While MotionWare has many attractive analysis features and commands (e.g. measuring sleep fragmentation, total sleep time, etc.), these are calibrated for humans, and even those studying humans suggest not to merely trust these pre-determined algorithms (Paquet *et al.*, 2007). For this reason, I only used this software to extract the raw data from accelerometers that export the raw data.

I omitted any loggers with saturated activity scores from the analyses, as this data was likely skewed by water damage, and non-skewed data could not be properly distinguished. To characterise the daily architecture of activity in slow lorises, I analysed accelerometer data using unsupervised validation via survival curve analyses and data simulation (see Criteria for Sleep section below) using Microsoft Excel and MATLAB version R2017b. I present all data as mean ± SEM when comparing all individuals, or as individual points when showing representative data from a single animal. Each data representation type is specified in the respective legend for each figure.

Although prolonged bouts of inactivity are likely to represent sleep (Kanady *et al.*, 2011; van Hees *et al.*, 2015; Samson *et al.*, 2016; Van Oort *et al.*, 2004), the possibility remains that some portions of inactivity are merely quiet immobile wakefulness. With careful analyses, accelerometers can be used to interpret sleep-wake patterns, although the least assumptive way to approach this would be through the production of an actigraphy with raw activity counts provided by the accelerometer. An actigraphy is a continuous measurement of activity levels across multiple 24-h cycles. Specifically, actigraphy record movement, which can later be further analysed to interpret circadian sleep/wake patterns or even disorders (Ancoli-Israel *et al.*, 2003; Sadeh *et al.*, 1995). An actigraph is the production of a measured representation of daily activity levels across multiple 24-h cycles. It is pertinent, however, to be particularly careful with detecting wake from sleep, as the accelerometers can often detect movement that is not necessarily exerted by the focal animal (e.g. shaking branches, wind, etc.). This limit should be considered when using supervised or unsupervised validation, but one must still be careful not to misinterpret the data.

While simultaneous behavioural observations can be used to extrapolate specific behaviours from activity scores, basic latent behaviors can be extrapolated from accelerometers using unsupervised algorithms for larger datasets or cryptic species [Brown *et al.*, 2013; Chimienti *et al.*, 2016; Sakamoto *et al.*, 2009). I used survival curve analyses and data simulation as methods of unsupervised validation, in estimating patterns of activity and inactivity of animals. I used activity scores to devise criteria for sleep, defining inactivity as complete immobility (activity score equal to zero). I used survival curve analyses as an approach to assess continuity of activity and rest bouts, and plotted the distribution of rest episodes during the night and during the day as a function of their

progressively increasing duration, expressed as a percentage of the total number of episodes. Survival curve analyses have been used, predominantly in captive research, to distinguish thresholds of data for analyses. In particular, survival curves examine the duration of which bouts of activity and rest episodes survive before termination (Klerman *et al.*, 2013). This analysis allows the plotting of data distribution as a function of their progressively increasing duration. Particularly in sleep research, researchers have found survival curve analyses to be successful in comparing the duration of sleep bouts in relation to subsequent bouts, in determining relationships (Klerman, *et al.*, 2013). I performed this same analysis for episodes of activity to quantify how long episodes sustained. To determine the duration of time for sleep, I performed survival curve analyses on each individual, comparing the duration of uninterrupted inactivity at thresholds of 0 min, 1 min, 2 min, 3 min, 4 min and 5 min. I performed non-parametric Wilcoxon Signed Ranks Tests to compare the mean proportion of time individuals spent in activity between the day and night, as well as the mean ambient temperature levels. I also compared ambient temperatures during bouts of activity/inactivity, and during the absence or occurrence of naps (see below, for nap analyses).

BRIEF AWAKENINGS

To determine if activity occurrences during the day represents brief awakenings from sleep, I used data simulation to measure the likeliness of immobility compared to chance. Data simulation is used to investigate the dynamics of space and time (Crawley, 2012), and is regularly used to measure EEG data to distinguish SWS within a sleep-cycle and determine sleep homeostasis in animals, including humans (Achermann & Borbely, 1990; Borbely, 1982a, 1982b; Franken *et al.*, 1991; Huber *et al.*, 2000). This method is being increasingly used in interpreting and analyzing bio-logging data measurements, from animal ranging behaviours to distinguishing sleep stages (Huber *et al.*, 2000). By comparing simulated

data to empirical data, one can distinguish the validity of their results, by measuring the likeliness of empirical data occurrences in comparison to randomized occurrence in simulated data. This is particularly useful for validating unsupervised accelerometer recordings, to limit potential for misinterpreting the output.

To determine if daytime occurrences of activity represented brief awakenings from sleep, I tested the likelihood of animals to remain immobile longer than could be expected by chance. To this end, I shuffled the timing of the occurrence of all brief awakenings with their corresponding durations randomly across the day to quantify if the same distribution of rest bouts is observed in both the original and simulated datasets (Figure 5.4).

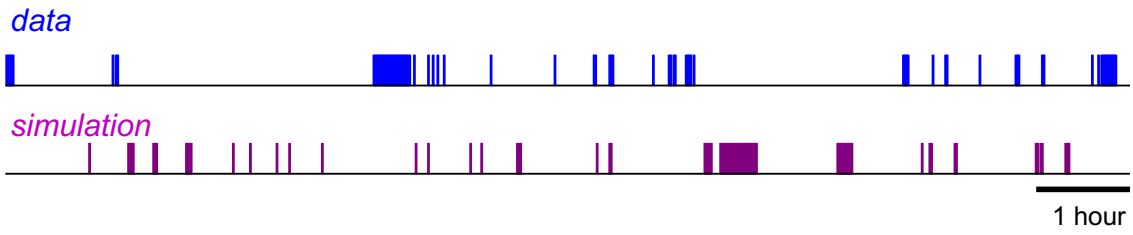


Figure 5.4. Representative profile of activity (where 1-min bins with activity>0 shown as 1s) across one light period between sunrise and sunset. The plot below shows reshuffled activity periods of the same recording, where the number and duration of short activity bouts are retained as above, but their timing of occurrence is randomised.

I additionally ran survival curves on empirical data and simulated data to determine the minimum duration of immobility without interruption that would most likely represent sleep. I performed Repeated Measures ANOVA tests to quantity the duration of brief awakenings and the duration of resting bouts across time, between sunrise and sunset, as a proxy for rest consolidation.

INTRINSIC SLEEP NEED

To test for homeostatic sleep regulation, I first measured the duration of locomotor inactivity as representative for rest consolidation during daytime rest, where least disruptions from rest represent higher sleep intensity. I additionally analysed the distribution of consolidated rest bouts over time and between individuals, to test if rest bouts are random. To test if naps influenced the preceding daytime rest, I compared individuals and their occurrence (or absence) of naps in relation to their subsequent sleep intensity during daytime rest. To determine if bouts of immobility during the night represent naps, I used data simulation to measure the likeliness of immobility compared to chance. To do this, I ran the same analyses stated above for quantifying brief awakenings, with bouts of immobility during nightly activity patterns.

5.3 RESULTS

While accelerometers were fitted to twelve individuals over the duration of this study, only seven of the retrieved accelerometers had complete data stored. I omitted any loggers with saturated activity scores from our analyses, as these data were likely skewed by water damage, and non-skewed data could not be properly distinguished. Of the retrieved complete loggers, I acquired accelerometer data on 4 females and 3 males, of which 6 were adults and one was a juvenile (female). Retrieved accelerometers contained data for an average of 46.3 ± 0.8 days per logger, contributing to a collective 321 days (46.3 ± 0.8 days per logger) of data between the individuals (Figure 5.5).

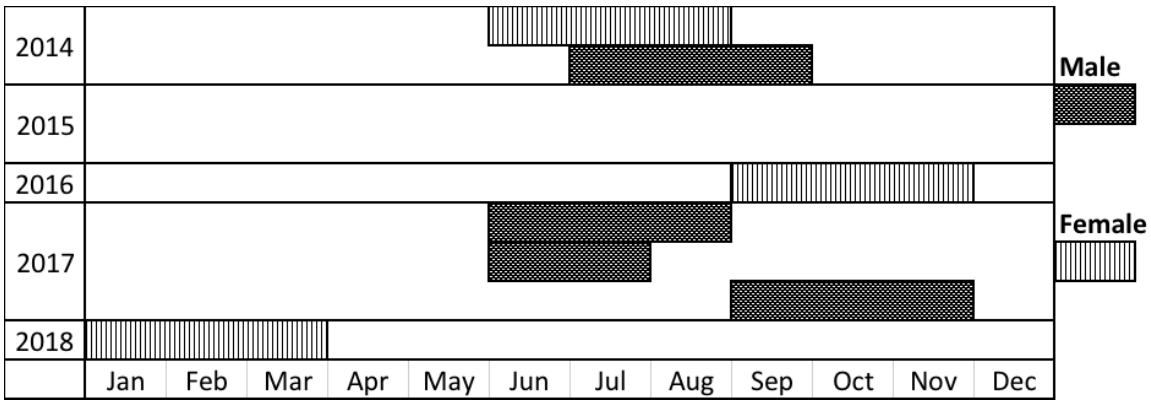


Figure 5.5. Timeline of retrieved accelerometer recordings on individual animals (n=7) shown separately for males and females. Each row represents one individual loris.

5.3.1. LIGHT ENVIRONMENT AND TEMPERATURE INFLUENCE

Over the duration of this study, the average time (hh:mm±SD) of sunrise was 05:49±00:12 while sunset occurred at 17:51 ± 00:31. I focused on activity versus inactivity to look at parameters of rest duration, onset and latency of rest, and rest disruption. The amount of activity individuals performed was significantly lower during the day compared to night, where a striking consistency was observed between days with respect to activity offset and onset. All animals displayed elevated continuous nocturnal activity pattern with prolonged periods of monophasic behavioural rest occurring almost exclusively during the day (Figure 5.6). The total daily amount of activity (defined as 1 min epochs with at least 1 activity count) and inactivity (defined as 1 min epochs with 0 activity counts) were on average similar (activity: 12.6±0.5, rest: 11.1±0.5 hours; Z = 1.521, p= 0.128), with periods of inactivity during the dark period being rare, and the reverse pattern displayed during the light period (Z = 2.3664, p= 0.0180).

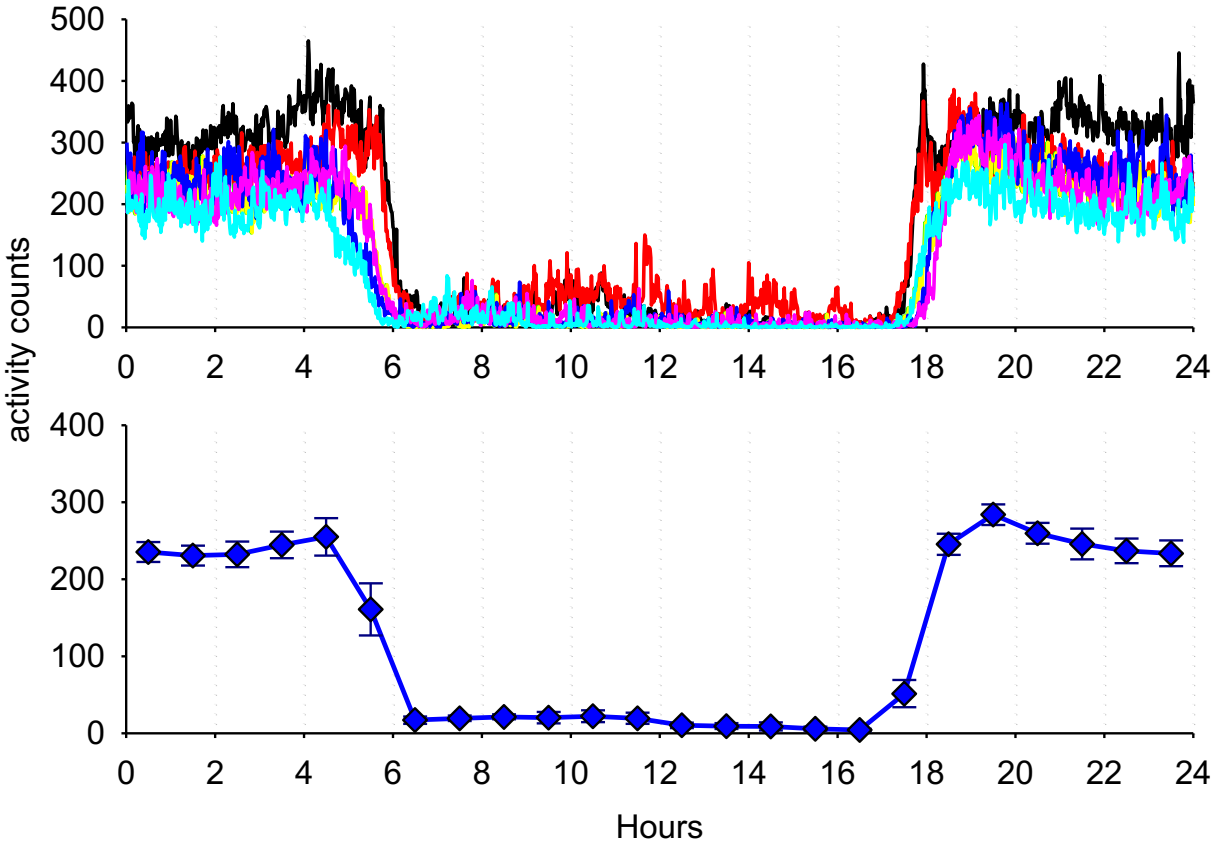


Figure 5.6. Top: average activity profile plotted for each individual loris over 24-hours; 1-min resolution. Each colour represents a different individual; bottom: average activity (SEM, n=7) shown in 1-hour intervals.

Changes in activity anticipated day-night transitions, where all animals typically displayed an onset and cessation of activity in relation to sunset and sunrise. Individuals began transitioning from active to inactive an approximately one hour prior to sunset, and transitioned from inactive to active within approximately 20 mins prior to sunset (Figure 5.7).

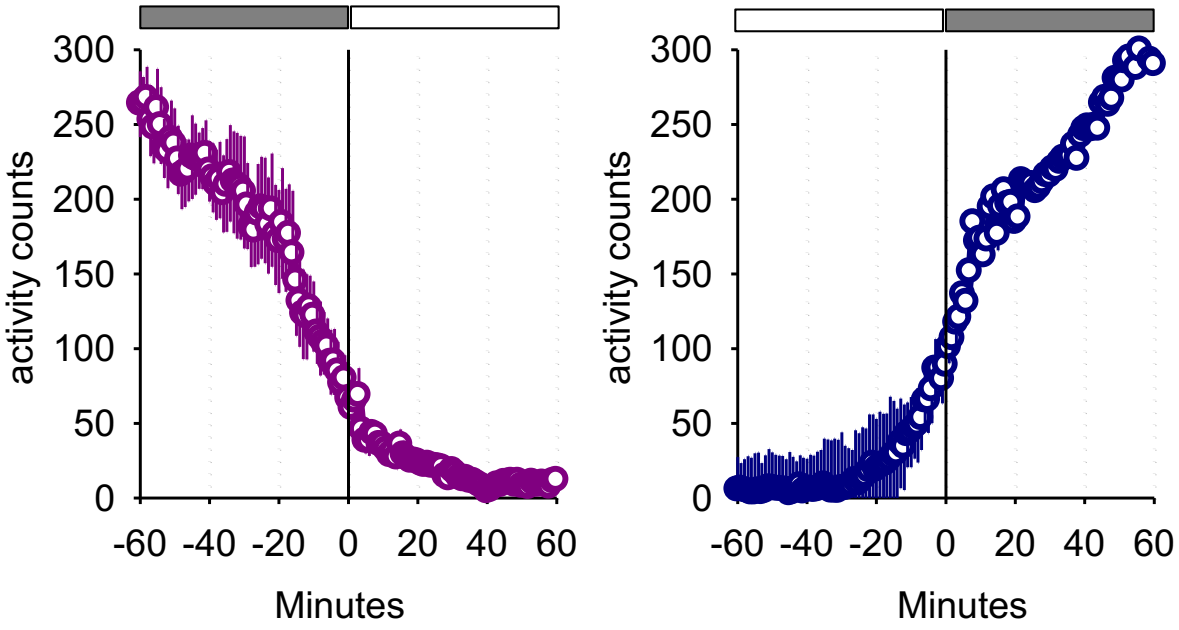


Figure 5.7. Left: The time course of locomotor activity at the transition from night to day (depicted as the bar above the curves). 1-min values of activity (first averaged between days within an individual, prior to calculating means between individual lorises, n=7, SEM) are plotted for one hour prior to sunrise and one hour after the sunrise. Right: Same as Left, for the day-night transition.

Ambient temperature values showed larger fluctuations during the day, while it was relatively stable during the night. During the day, ambient temperature displayed a mean value of 21.28 ± 0.92°C, and a mean value of 16.90 ± 0.60°C at night ($Z = 5.905$, $p < 0.001$). The daily time course of temperature reached an average minimum around sunrise (15.33 ± 1.85°C), while mean temperature reached an average maximum at midday (21.27 ± 0.39 °C; Figure 5.8).

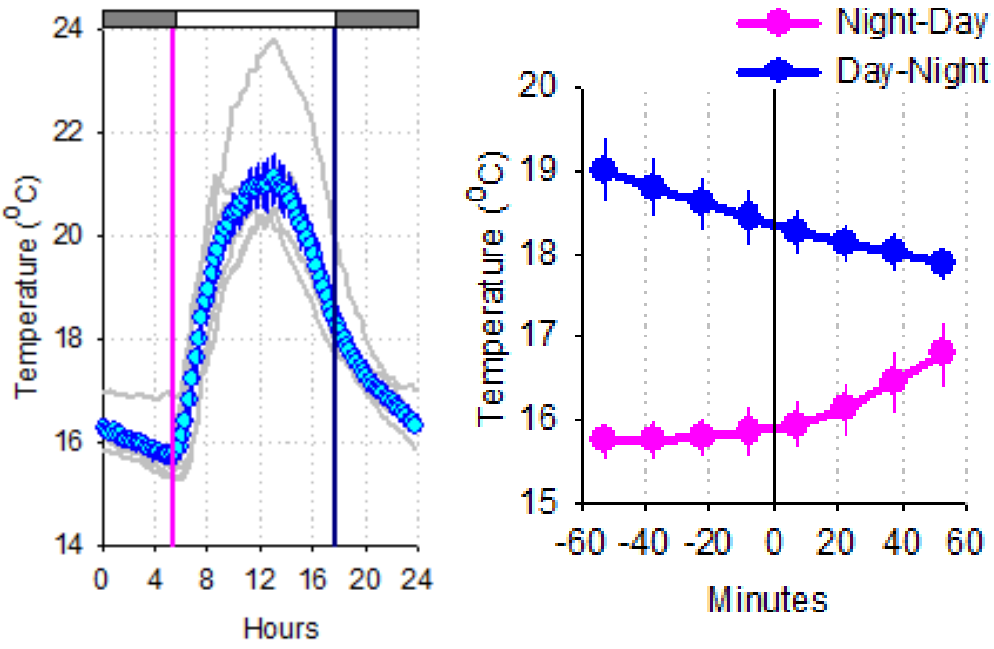


Figure 5.8. Left: The time course of average ambient temperature recorded from all individual lorises (n=6, SEM). 15-min values are plotted consecutively from midnight till midnight next day. Vertical lines depict average time of sunrise (magenta) and sunset (dark blue). Right: Time course of ambient temperature during the corresponding 2-h intervals as shown in Figure 5.6. Mean values (n=6) are plotted in 15-min intervals.

5.3.2. SLEEP AND RESTING PATTERNS

Across 24-h, sustained periods of activity lasted significantly longer than sustained periods of rest, suggesting that sustained immobility for longer than approximately 2-h was extremely unlikely (Z = -2.366, p = 0.016). Quantification of the number and duration of brief arousals occurring during the day revealed that these events occur on average 1-2 times per hour and mostly last < 5 min (Fig 5.9; $F(11, 66) = 6.45$, $p < 0.001$).

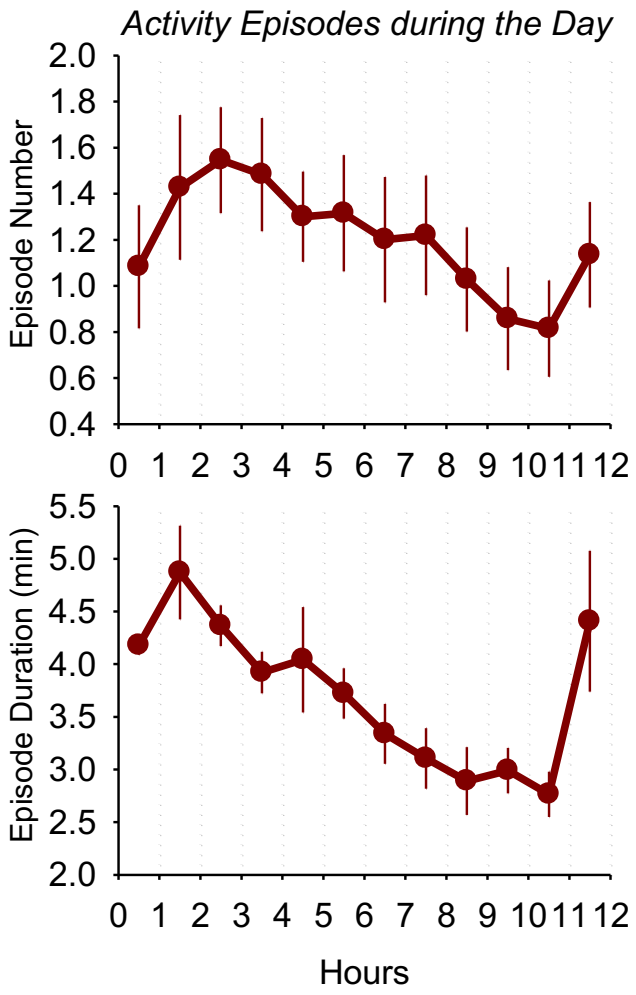


Figure 5.9. Top: time course of activity episode number during the day. The values are plotted in 1-h intervals from sunrise to sunset. Mean values, n=7. Bottom: time course of activity episode duration during the day. The values are plotted in 1-h intervals as above.

While the occurrence of brief awakenings is an important feature of physiological sleep, they could also represent movement episodes occurring randomly during resting wakefulness (Franken *et al.*, 1991). Using this data simulation, I found that long periods of inactivity were rarely encountered with an artificial time series.

Visual inspection of the resulting time series, suggested that placing brief awakenings at random often resulted in a premature termination of prolonged rest bouts encountered in the original activity plots. To quantitatively assess this observation, I plotted the distribution of rest bout durations as a function of their duration for both the original and simulated data set, which revealed a slightly lower probability of the occurrence of long rest periods above approximately 30 min in the simulated time series. In other words, the simulated dataset was more likely to contain short rest periods where rest bouts lasted <30 minutes, but less likely to contain longer rest bouts (Figure 5.10, left), indicating that rest bouts detected with actigraphy do not occur randomly between movement episodes, and thus may represent episodes of consolidated sleep. This effect was attenuated, when interruption criteria were introduced (Figure 5.10, middle). Mean duration of rest periods do not differ between data and data simulation, suggesting that the timing of occurrence of brief arousals contains important information about rest consolidation, beyond merely rest episode duration, which was not different between the original and the simulated data sets (Figure 5.10, right).

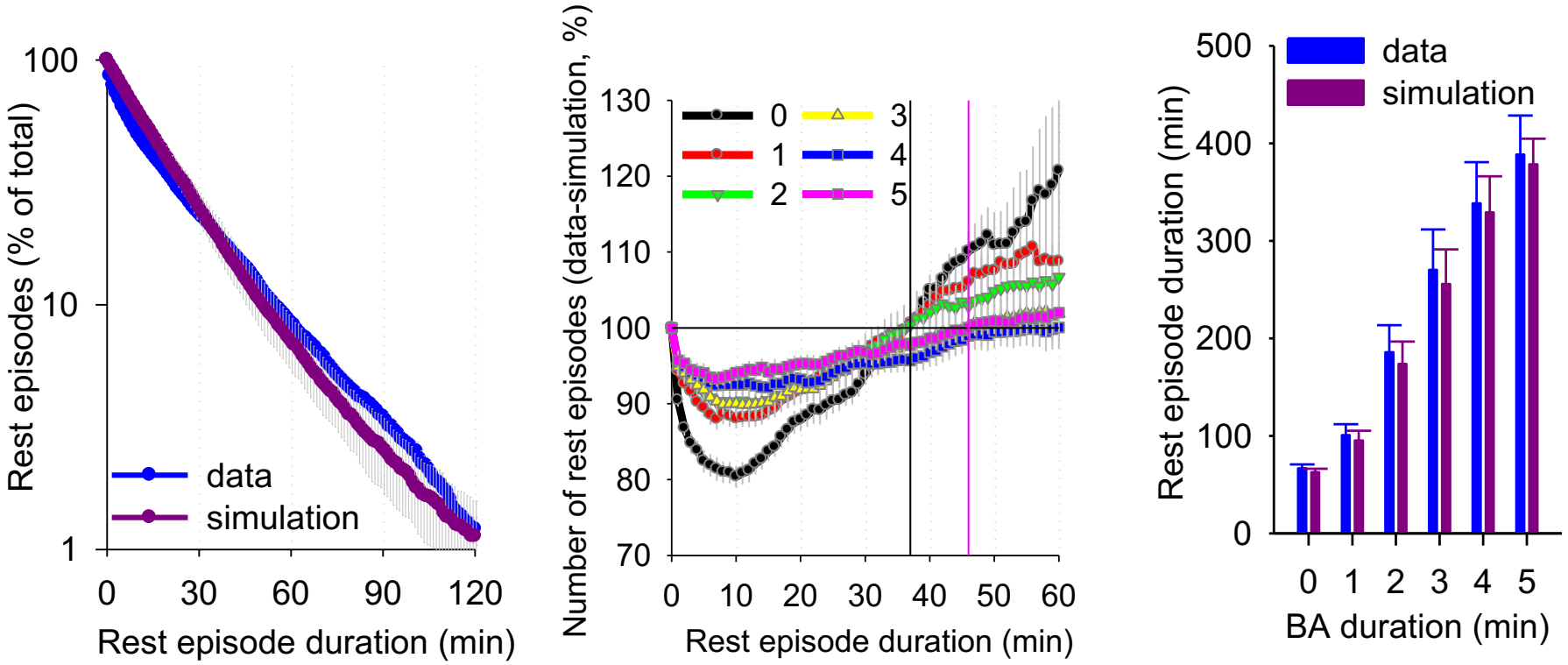


Figure 5.10. Left: Survival analysis of inactivity (rest) episodes for empirical data and simulated activity profiles as above. Note that shorter rest bouts are more likely to occur in the simulated activity time profiles, while longer sustained rest periods occur more frequently in the real data; Middle: The difference in the distribution of rest episodes derived from data and simulation, calculated as in left survival analyses. An additional interruption criterion is introduced from 0-5 min. Vertical lines depict minimal rest episode duration where the rest episode duration in data were below simulation for rest episodes where no interruption is allowed (black) and where up to 5 min brief awakenings are permitted (magenta). Right: Mean duration of rest episodes derived from empirical and simulated data sets, shown as a function of the interruption criterion. Mean values, n=7, SEM

5.3.3. SLEEP HOMEOSTASIS

The key characteristic of physiological sleep is its homeostatic regulation, which is best represented by the levels of EEG slow-wave activity and the occurrence of consolidated periods of sleep or rest, less frequently interrupted by brief awakenings (Franken *et al.*, 1991). Calculating the occurrence of rest episode duration between the sunrise and sunset revealed that rest consolidation varied significantly over the course of the day, with longer rest episodes and lower incidence of brief awakenings at the beginning and towards the end of daytime rest (Fig 5.11; $F(11, 66) = 4.64$, $p < 0.001$).

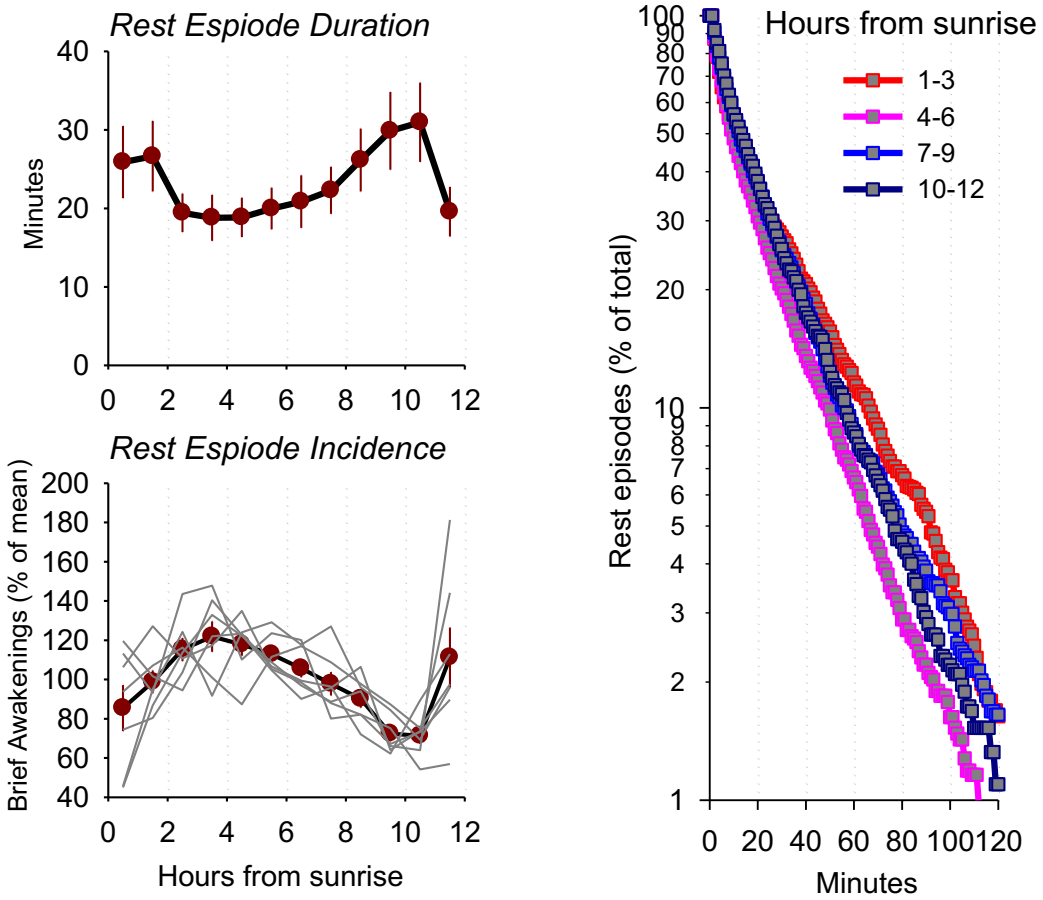


Figure 5.11. Top Left: Time course of rest episode duration across the light period. Mean values (n=7, SEM) are shown as dark red symbols; Bottom left: Time course of rest episode number across the light period. Mean values (n=7, SEM) are shown as dark red symbols. The values are expressed as percentage of mean across the entire day. The curves for individual animals are shown in grey lines; Right: Survival analysis of inactivity (rest) episodes shown for 3-h intervals across the day. Note that rest episodes "survive" for longer at the beginning of the day, then their duration drops and tends to increase again in the second half of the light period. Mean values, SEM, n=7.

To directly assess the relationship between ambient temperature and rest episode characteristics, I clustered all rest episodes based on the average temperature during their occurrence, which revealed a positive association. Specifically, rest duration was longer when T_a raised above 13°C (Fig 5.12 left). Temperature change within rest episodes also mattered, with shortest episodes corresponding to stable temperature levels, but increasing along the steep upward and downward shifts in temperature (Fig 5.12 right).

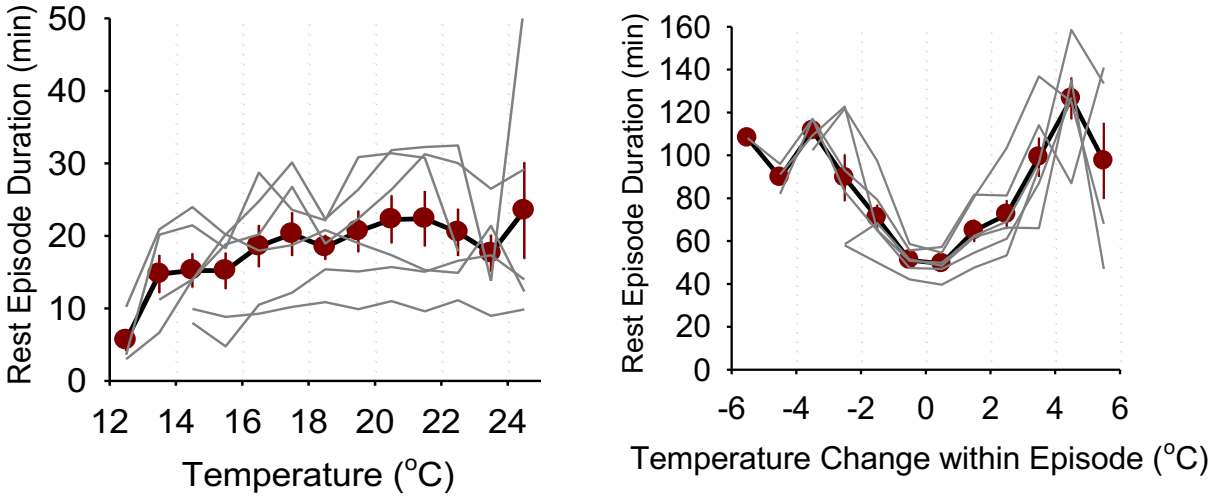


Figure 5.12. Left: The relationship between ambient temperature and rest episode duration during the day. All rest episodes during the day are grouped according to the ambient temperature at the time of their occurrence. Note that at warmer temperatures the rest episodes tend to be longer. Right: The relationship between change in ambient temperature within sustained rest episodes and their duration. All rest episodes are grouped according to the magnitude of change in temperature, and the corresponding values are averaged. Note that rest episodes tend to be longer if the temperature is decreasing or increasing, but remain short if the temperature is stable. Mean values, n=7, SEM.

Although the majority of night time activity periods were uninterrupted, the occurrence of consolidated periods of total or partial inactivity was not uncommon, and was encountered in all animals. While it is unknown whether such episodes of inactivity represent merely wakeful immobility or sleep, I tentatively referred to periods of immobility of ≥10 min as putative “naps”. All animals displayed varying quantities and duration of naps in their regular activity patterns, ranging approximately between 10-60 mins, with some individuals napping almost daily and others only occasionally (Figure 5.13, left). Looking at activity levels surrounding naps, in some cases a marked surge of activity prior to a nap but it was more common that an activity increased transiently in the first 5-10 min after naps (Figure 5.13, top). As the occurrence of naps during the night may reflect increasing sleep pressure, I calculated the timing of nap occurrence, which revealed that it was highly unlikely that an individual displayed a nap during the first few hours after sunset, while the probability of napping increased substantially towards the mid-portion of the night (Figure 5.13, right).

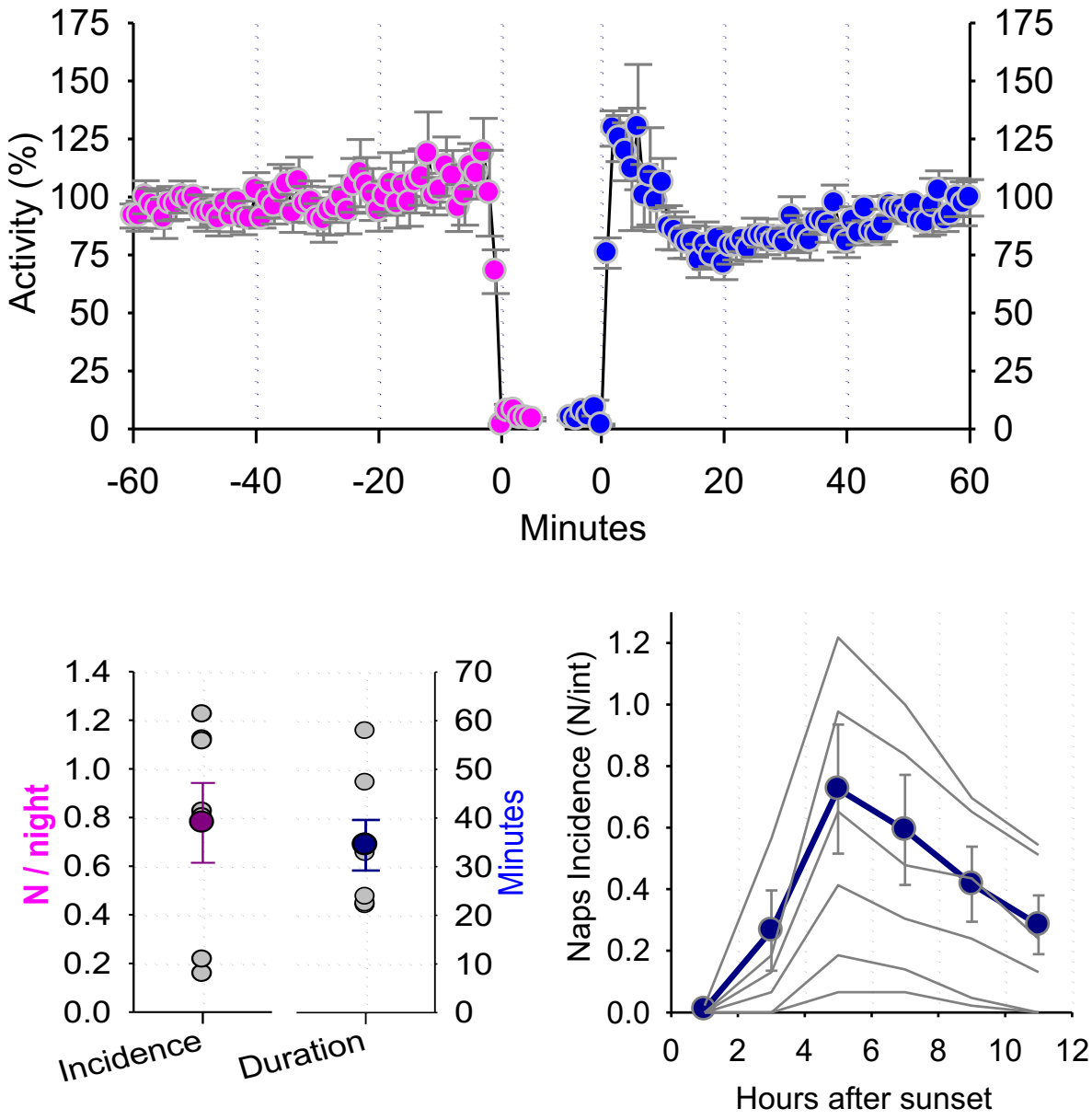


Figure 5.13. Top: The time course of activity around the nap. All naps lasting at least 10 min are aligned to their onset and offset and activity is averaged in 1-min bins over 1-h period before and after the nap. Note a surge of activity immediately after the nap. Left: Mean values of nap incidence and duration during the night across the entire recording period. Mean values, SEM are shown as coloured symbols and individual animals (n=7) are shown in grey. Right: The time course of nap incidence during the night. All naps lasting at least 10-min were detected across the entire recording period, and grouped according to the timing of their occurrence between sunset and sunrise. Note that the probability of nap occurrence is low during the early hours of the night, but increases progressively towards the middle of the night, and then decreases prior to morning hours.

5.3.4. ENVIRONMENTAL TEMPERATURES AND NAPS

To test the relationship between T_a and nap onset, I examined the occurrence of naps (minimum: 1) compared to the absence of naps in relation to subsequent T_a. The levels of T_a did not show a strong association with the occurrence of naps (Figure 5.14 left; Z= -1.363, p= 0.173). To further test for sleep homeostatic regulation, I tested if the occurrence of napping influences activity patterns during preceding daytime rest, in contrast to animals that were continually active during nightly activity. However, the occurrence of naps during the night was unrelated to the amount of activity during the preceding light phase (Figure 5.14, right).

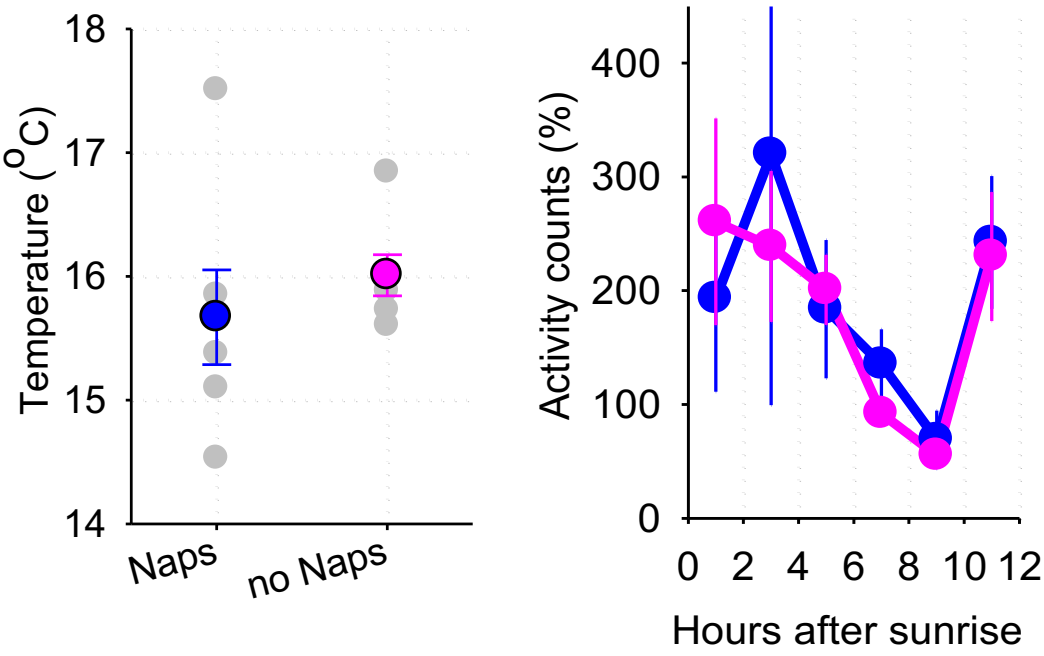


Figure 5.14. Left: The relationship between the occurrence of naps and ambient temperature. For each night across the entire recording period the occurrence of naps was determined and subsequently corresponding mean nightly temperature values were calculated separately for the night with at least one nap, and those nights where no naps occurred. Mean values, SEM are shown as coloured symbols and individual animals are shown in grey.; Right: Time course of activity during the days following those nights where the animals took naps (blue curve) and those nights where the animals were continuously active. 2-h mean values (n=7, SEM) of activity are represented as percentage of mean activity during the preceding day.

Light environment can synchronize a mammal's circadian clock, regulating both behavior and sleep (Peirson *et al.*, 2018). This synchronization is due to retinal photoreceptors and their photosensitivity in responding to light cues, commonly referred to as Zeitgeber. Circadian rhythms allow an animal to predict regular changes in its environment, such as sunrise and sunset (Fisk *et al.*, 2018). *Nycticebus javanicus* displayed circadian rhythms that were highly synchronized with the light environment, anticipating sunrise and sunset. As ambient temperature transitions between the day and night were modest and typically did not exceed 1 or 2°C, it can be further surmised that transition of activity patterns are more likely driven by either anticipation of day and night and the animals' intrinsic circadian clock or by changing levels of light, suggesting that in this species the circadian occurrence of activity and rest is strongly entrained to the periodicity of day and night.

In addition to activity transitions, levels of light and daily fluctuations of ambient temperature played a role in overall resting patterns. It has been reported in endotherms (including humans) that sleep and circadian rhythms can be shifted when an individual is exposed to temperatures exceeding one's thermoneutral zone (Cirelli *et al.*, 2004; Heller *et al.*, 1983; Kowalczyk *et al.*, 2003; Lima *et al.*, 2005; Manzar *et al.*, 2012). We observed that slow lorises experienced elongated resting bouts during their daytime rest in response to warmer ambient temperatures. The strong effects of temperature on sleep in slow lorises have important implications, as existing populations of *N. javanicus* are constantly being forced into high altitude habitats as a result of agricultural expansion and deforestation (less than 9% of forest remains on the island of Java). At higher altitudes, temperature variation increases (Dunbar, 2002) which would likely affect their sleep consolidation. Thus, if populations continue to be pressured into higher elevational gradients, they will likely encounter lower sleep intensity.

Mammals and birds display homeostatic sleep regulation, where prolonged wakefulness is followed by prolonged rest during subsequent sleep (Jones *et al.*, 2008; Siegel, 2003; Tobler, 1985). Similarly, animals display sleep rebound where sleep is significantly disrupted mid-rest or when there is a loss of sleep (sleep deprivation) altogether (Rechtschaffen & Bergmann, 2002; Vyazovskiy *et al.*, 2007). This observation is consistent with previous studies in laboratory animals and humans, whose sleep is frequently punctuated with brief arousals (Borbély 1984; Lo *et al.*, 2004; Mattmann *et al.*, 1982; Penzel *et al.*, 2004; Winnebeck *et al.*, 2018; Yetish *et al.*, 2018). This rebound is acquired at a later period (such as during regular active periods), or extended on to the subsequent sleep. *Nycticebus javanicus* displayed prolonged immobility at the beginning of daytime behavioral rest, and this pattern suggests that animals experience a deeper sleep in the first portion of behavioral rest, following a period of activity, consistent with other studies in humans and laboratory animals (Vyazovskiy & Delogu, 2014; Winnebeck *et al.*, 2018).

Researchers have hypothesized that less disturbances during sleep increases the intensity of REM (Rapid Eye Movement) and NREM (non-REM) sleep, which is referred to as the "sleep quality hypothesis" (Febinger *et al.*, 2014; Leys *et al.*, 2012; McGrew, 2004; Samson, 2013). Comparative research on baboons and orang-utans found that sleeping structures that promote specific sleep postures increase sleep quality (Samson and Shumaker, 2015). A previous study on sleeping site selection on the population we studied found *Nycticebus javanicus* to select bamboo almost exclusively as sleep locations (Nekaris *et al.*, 2017). Lorises display unique vascularization of their forelimbs that allow them to hold substrates for prolonged periods of time without their hands going numb. Thus, in slow lorises the structure and size of bamboo as a sleeping substrate is in accordance with the sleep quality hypothesis, aiding undisturbed sleep (McGrew, 2004;

Samson, 2012; Samson and Shumaker, 2015). We also observed that *Nycticebus javanicus* displayed a higher incidence of naps towards the middle of the night—their usual active period. As there was no indication that the occurrence of naps was triggered by changes in ambient temperature or light levels, it is possible that this reflects increased sleep propensity, indicative of higher sleep need accumulated as a result of continuous activity. However, the occurrence of these periods of inactivity during the night was unrelated to the amount of activity during the preceding day, suggesting that extrinsic factors are an important determinant of napping, above and beyond the intrinsic sleep need.

Nycticebus javanicus displayed highly consolidated monophasic sleep patterns. Sleeping at distinct times of day (in this case, dawn until dusk), *N. javanicus* conformed to typical patterns of a nocturnal, monophasic mammal (Ball, 1992; Lima *et al.*, 2005; Tobler, 1989). Most non-human primate species display monophasic sleep patterns (Lima *et al.*, 2005) with polyphasic sleep patterns typically exhibited by small-bodied rodents and insectivorous mammals (Lima *et al.*, 2005). These tendencies are suggested to be both largely due to the metabolic processes unique to sleep, when protein synthesis occurs (Cirelli *et al.*, 2004), as well as tendencies to be prey to many predators where periodic wakefulness can increase predator avoidance (Allison & Cichetti, 1976).

Researchers have reconstructed polyphasic sleep patterns to be an ancestral trait in mammals (Capellini *et al.*, 2008; Lima *et al.*, 2005), and have suggested that monophasic sleep is a derived trait in the anthropoid primates (Nunn *et al.*, 2010). It is still unknown, however, if this trait derived at the base of the primate order "with subsequent reversals in some strepsirrhines" or in the common ancestor of monkeys and apes (Nunn *et al.*, 2010). This holds true for primates, with a few exceptions. The cathemeral primates display

polyphasic sleep patterns during both the day and night, similar to ancestral mammalian sleep traits (Curtis & Rassmussen, 2006; Nunn *et al.* 2010; Santini *et al.*, 2015; Tattersall, 1987, 2006). Mouse lemurs (*Microcebus* spp.) also display tendencies towards exhibiting polyphasic sleep patterns. Mouse lemurs, owl monkeys and cotton top tamarins are considered marathon sleepers, displaying an average total sleep time of 13 to 17 hours, with owl monkeys and mouse lemurs being the two species of primate known to sleep the longest (Nunn *et al.* 2010). For monophasic sleepers, the nocturnal species studied so far have displayed longer sleep duration than diurnal species.

Monophasic sleep is suggested to be more efficient because it involves more time in deep sleep, thus requiring a lower total sleep time per day to meet sleep requirements (Capellini *et al.*, 2008; Nunn *et al.*, 2010). Our finding that slow lorises perform a relatively (to other nocturnal primates) shorter total sleep duration, however, contradicts the assumption that diurnal primates have evolved to require less total sleep time, as a result of increased sleep intensity. Overall, *N. javanicus* displayed activity patterns that are strikingly synchronized with sunset and sunrise and rest fragmentation and duration that are correlated to temperature changes. Our results urge more research on the sleep patterns of other wild mammals, particularly primates, in testing hypotheses on sleep traits and how they may be influenced by changes in the natural environment.

5.5. CHAPTER SUMMARY

This chapter represents the first to describe and measure behavioural sleep (rest) of a wild nocturnal primate the Javan slow loris *Nycticebus javanicus*. In this chapter, slow lorises displayed generally similar duration of immobility-defined sleep as diurnal primates, maintaining an average of eleven hours of sleep on a daily basis. The most important novel finding was that environmental variables, such as the levels of light and ambient temperature had major influence, shaping the overall pattern of activity and rest across 24 h. Increased consolidation of rest at the beginning of their habitual sleep period may reflect increased sleep 'intensity' or direct influence of ambient temperature. This study therefore raises an intriguing question of how wild animals cope with obtaining sufficient sleep, or compensate for sleep loss incurred during spontaneous or enforced wakefulness while adjusting their sleep pattern to predictable or unpredictable fluctuations in the environment, particularly regarding thermoregulation and temperatures diverging from their TNZ.

CHAPTER 6. BEHAVIOURAL FLEXIBILITY OF *NYCTICEBUS JAVANICUS* IN A HABITAT WITH NON-ENDIGINOUS PLANTS (*CALLIANDRA* SPP.)

Figure 6.1 has been removed from this version of the thesis due to copyright restrictions

Figure 6.1. Photograph taken with external flash of a wild Javan slow loris (*Nycticebus javanicus*) foraging on *Calliandra calothrysus*, in Cipaganti, West Java. Photo by S Anza.

6.1. INTRODUCTION

The introduction of non-indigenous taxa into a foreign environment can jeopardise levels of biodiversity, having a negative impact on the function of an ecosystem (McCarthy, 1997; Thompson *et al.*, 1987). In particular, the introduction of invasive alien plant species can lead to the extinction of native species, as well as decreasing or terminating resources used by endemic animals (Blossey, 1999; Braithwaite *et al.*, 1989). Conversely, in the last decade, researchers have discovered situations where introduced plant species have provided viable and sustainable refuge and food source alternatives for native vertebrates (Eppley *et al.*, 2015; Gérard *et al.*, 2015). Deliberate introduction of non-native (but often closely related) taxa is a conservation method called rewilding, used to restore and manage ecosystems in nature (Donlan *et al.*, 2005; see section 1.4).

To determine if an invasive alien species should be controlled, removed or left to remain, the COP has devised a number of necessary assessments, including: the vulnerability of habitats/ecosystems to invasion by alien species; the impact of alien species on biological diversity; the various pathways for the introduction of invasive alien species; and the socio-economic implication of invasive alien species on for indigenous and local communities (COP 6 Decision VI/23c *'Alien species that threaten ecosystems, habitats or species'*).

Information on the impacts of non-indigenous taxa across Southeast Asia are predominantly anecdotal observations with virtually no measurements of effect (Peh, 2010). Compared to other tropical regions, Southeast Asia is reported to have experienced the most severe biodiversity detriments to its ecosystems due to climate change, anthropogenic land changes and overexploitation, and is predicted to continue towards a biodiversity crisis (Estrada *et al.*, 2017; Peh, 2007, 2010; Sodhi *et al.*, 2004). The countries of Southeast Asia belong to diverse levels of political and economic development,

demanding attention towards the management and effects of invasive species introduced to the region, and between countries to monitor any biodiversity loss or success.

While multiple forest habitat types historically characterised the Indonesian island of Java, centuries of intensive agricultural expansion have now transformed forested area into mosaic agroforestry landscapes (Whitten *et al.*, 1996 ; Nekaris *et al.*, 2017; Reinhardt *et al.*, 2016). Ten species of *Calliandra* originating from Guatemala had been introduced to Indonesia through the Bogor Botanic Gardens in West Java, in 1936 (Breteler, 1989; Macqueen, 1996). *Calliandra* is a plant genus from the Leguminosae family (commonly referred to as synonym, Fabaceae), with a total of 132 species, predominantly deriving from Central and South America, with a few originating in continental Africa, Madagascar, North America and subcontinental India (Breteler, 1989; Riswan *et al.*, 1996; Thulin *et al.*, 1981). *Calliandra plants* are most used in West Javan agroforestry systems for fuelwood, intercrop hedgerow, plantation shade, soil stability and soil fertility (MacQueen, 1993, Riswan *et al.*, 1996).

Calliandra species produce varying quantities of nectar, which is consumed by several species of flying and non-flying mammals, including primates (Carthew & Goldingay, 1997). Nectar is high in sugar, water and amino acids, providing an important food and water source for many species and attracting pollinators (Johnson *et al.*, 2001). A limit or absence of potential pollinators is considered to be the most influential factor on fruit set of alien plant species introduced to new ecosystems (Chamberlain & Rajaselvam, 1996).

Lorisinae have regularly been observed to feed on floral nectars, sometimes displaying more than one third of their diet (range: 2-41%) to be composed by this food source:

Nycticebus bengalensis, N. coucang, N. javanicus and N. pygmaeus (Cabana et al., 2017; Gochman et al., 2016; Moore, 2012; Rogers & Nekaris, 2011; Starr & Nekaris, 2013; Swapna et al., 2010) . The Javan slow lorises have been observed to regularly consume nectar from *Calliandra* plants in West Java, Indonesia (Cabana *et al.*, 2017; Moore, 2012). As *Calliandra* spp. have been introduced abundantly across the island of Java over the past ~80 years, it is particularly important to understand the role these plants have filled in an ecosystem with threatened endemic taxa(such as the Critically Endangered *N. javanicus*) and how native taxa have adapted to its introduction.

In this chapter, I investigated the prevalence of one genus of introduced non-native plants in supporting native taxa and sustaining an ecosystem as per COP guidelines (see General Introduction, section 1.4). In particular, I investigated how introduced *Calliandra* spp. influenced the behavioural ecology of *N. javanicus,* and if they could be a potential ecological substitute in re-wilding practices for ecosystem restoration. I made the following predictions: i.) *Calliandra* spp. would produce flower and fruit throughout most of the wet season, when *Calliandra* spp. tends to bloom; ii.) *N. javanicus* would feed regularly on *Calliandra* plants nectar, suggesting it is a suitable food source substitute for a threatened taxa in this ecosystem; ii.) multiple native animal taxa (including *N. javanicus)* would display foraging behaviours and re-visiting to *Calliandra* plants that suggest a large native pollination network; iv.) native wildlife taxa would have a positive influence on fruiting and flowering of introduced *Calliandra* spp., suggesting plant-pollinator mutualism between introduced flora and native fauna. Based on these relationships, I wanted to assess the cost-benefit of *Calliandra* species as a non-native re-wilding tool for conservation planning in agroforestry ecosystems in Java where *N. javanicus* populations are present.

In Cipaganti, West Java (see section 2.2) *C. calothrysus* and *C. tetragona* (synonymous to *Zapoteca tetragona*) have been abundantly planted by local farmers throughout an agroforestry system, much like the rest of Java. Interestingly, Backer and Bakhuizen van Brink summarised cultivation reports of *Calliandra* spp., and many reports do not have record of *C. calothrysus* as being cultivated on Java (1963). It was concluded that the original introduction of *C. calothrysus* in Bogor was likely due to a misidentification of this species as *C. tetragona,* where both species regularly appeared and disappeared on the cultivation list, replacing one another's location during each consecutive report since 1941 (Backer & Bakhuizen van Brink 1963). Since the time of this discrepancy*, C. tetragona* has been revised as a new genus, *Zapoteca tetragona* (Hernandez, 1986), which is often used synonymously. Hereafter, I will refer to *Z. tetragona* as *C. tetragona*. *Calliandra calothrysus* and *C. tetragona* are very similar in their general height, leaf composition and pod shape, although they have distinctly different floral colours (Riswan *et al.*, 1996). *Calliandra calothrysus* produces deep red coloured flowers, while *C. tetragona* produces light white coloured flowers (Figure 6.2).

Figure 6.2. Top: *Calliandra tetragona (*synonymous with *Zapoteca tetragona)*; Bottom: *Calliandra calothrysus.* Photos by KD Reinhardt.

6.2.1. CALLIANDRA PHENOLOGY

To determine nectar availability of *C. calothrysus* and *C. tetragona* in Cipaganti, I monitored the flower phenology of both species (see section 2.3.3). I monitored phenology over the duration of 16 months, from June 2015 until November 2016.

For successful pollination to take place, pollen has to be transferred by the animal from the petiole of one flower to the stigma of another, during a non-destructive feeding bout. Obtaining evidence of this process is notoriously difficult, and is further hindered because many of the species involved are nocturnal and cryptic. However, continuous recordings of visitors to flower and pollen on a plant can be performed to detect what species contribute to the visitation network, and thus, pollinator community in an ecosystem (Lopezaraiza-Mikel *et al.*, 2007). Therefore, to gain more conclusive evidence, I used a combination of methods to compare the phenology of *Calliandra* plants in relation to re-visiting animals as well as the behavioural ecology of *N. javanicus* in identifying the pollinator community for this plant in this particular ecosystem.

6.2.2. FLORAL NECTAR FEEDING AND FORAGING TECHNIQUE

Using radiotelemetry (see section 2.3.1), I conducted nightly behavioural observations on *N. javanicus* using a combination of 5-min instantaneous sample points (see section 2.3.2) and recordings of all-occurrences of grasping behaviour during observed foraging and feeding behaviours (Altmann, 1974; Poindexter *et al.*, 2018). During observations of *N. javanicus* individuals, I recorded the food items and quantity consumed (Table 6.1) as well as the foraging technique used for food acquisition (Table 6.2).

Table 6.1. Food items consumed by *Nycticebus javanicus* and the units used to measure the amount consumed.

Food Item	Feeding Amount
Nectar	No. of seconds
Gum	No. of seconds
Fruit	No. of fruits
Insect	No. of insects
Vertebrate prey	No. of vertebrates
Leaves	No. of leaves
Bamboo shoots	No. of shoots
Flowers	No. of flowers
Other	No. of items

Table 6.2. Feeding techniques used by *Nycticebus javanicus* and the accompanying definitions.

Feeding Technique	Definition
Mouth	When only the mouth is used to consume food
Gouge	When individuals scrape bark to consume exudate foods
Unimanual grasp	When only one hand is used for grabbing food
Bimanual grasp	When both hands are simultaneously used for grabbing food
Reel	When both hands are used to grab food, passing the item off from one hand to the other (alternatively)
Other	When an infrequent foraging technique is used to acquire/grab food items

6.2.3. INVESTIGATING THE POLLEN NETWORK

As floral visitor recordings should be conducted when flowering peaks occur, I conducted all-night tree observations between March and May 2016, when flowers of *C. calothrysus* were in full bloom. To identify floral visitors and investigate the pollen network, I observed animal activity in individual trees (n=9) from dusk until dawn (18:30 until 06:30), as *Calliandra* is receptive to fertilization at night (Macqueen, 1993).

Mediating pollinator interactions is a method used to isolate specific pollinators from floral visitation to plants, and can be used to quantify a pollinator's influence on a particular species. (Forup & Menmott, 2005; Lopezaraiza-Mikel *et al.*, 2007). These methods are used most often to investigate the influence of an introduced or invasive species on the pollination network in an ecosystem (Gibson *et al.*, 2006; Kunin & Iwasa, 1996; Lopezaraiza-Mikel *et al.*, 2007).

To test for whether self-pollination occurs, flowers (when in the bud stage) or inflorescences can be placed in pollination bags, preventing outcrossing via pollinators or wind (Meyer, 1998). After isolating the flowers or inflorescences, one can record the subsequent phenology, focusing on fruit and seed production. Similarly, Chamberlain and Rajaselvam (1996) created an experimental design to determine the influence of pollinators on seed production, by controlling the access of different pollinators (Figure 6.3).

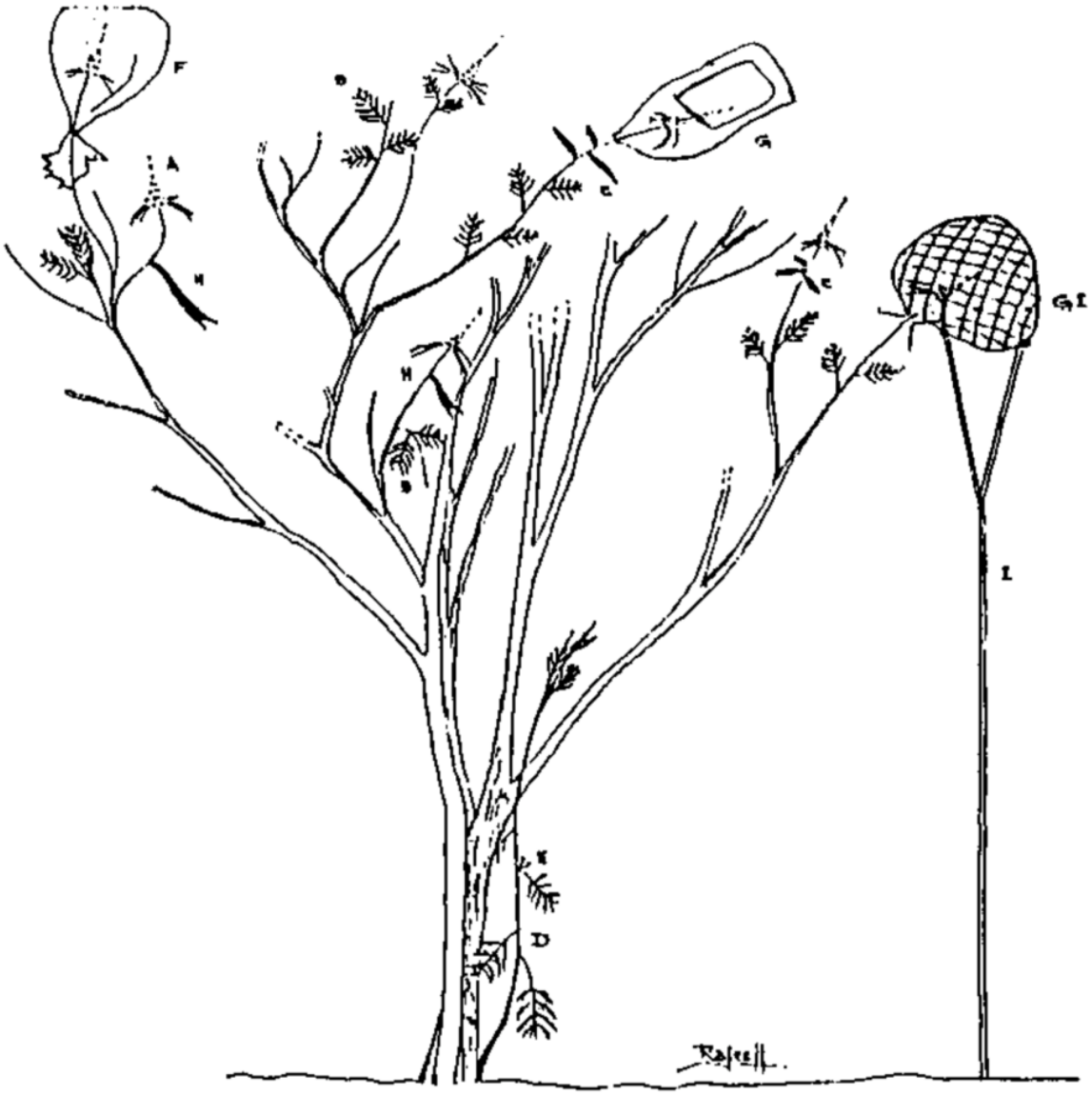


Figure 6.3. Experimental design for testing for pollinators of *Calliandra* spp. Figure from Chamberlain & Rajeselyam (1996), showing pollen proof bags (F), pollination experiment bags for artificial pollination (G) and bird netting to exclude flying and non-flying birds and mammals (GI).

Between June 2015 and August 2016, I performed isolation and pollination experiments to determine what species are potential pollinators of introduced *Calliandra* spp. plants in Cipaganti, using modified experimental designs modified from Meyer (1989) and Chamberlain & Rajeselyam (1996). To test for self-pollination, I placed pollen-proof bags on *C. calothrysus* (n=8) and *C. tetragona* (n=8) flowers at the bud stage, excluding

pollinators and outcrossing (see Figure 6.3, F). To isolate *Calliandra* spp. from flying and non-flying mammals, I encased *C. calothrysus* (n= 8) and *C. tetragona* (n= 8) inflorescences in 'isolation boxes' (Figure 6.4). I constructed isolation boxes by building a wooden box frame (L x W x H: 381 mm) with a hinged door, where each side was covered in bird netting (15 mm diamond mesh), secured with zip-ties. I notched the frame of the hinged door to accept the stem, allowing the entire inflorescence to fit inside the box without breaking the stem. I drilled the notches in the field to customise the fit for each plant, minimising space between the stem and notch edges.

Figure 6.4. A modified method from Chamberlain & Rajeselyam (1996) to control access of flying mammals and birds. These structures represent 'isolation boxes' to determine the role insects play in the pollination network. Isolation boxes are covered in 15mm diamond mesh bird netting and are fitted around *C. calothrysus* inflorescences. Photos by KD Reinhardt.

To further determine the role of non-flying mammals as pollinators, I isolated *C. calothrysus and C. tetragona* plants using constructed aluminium sleeves around the base of the plant (Figure 6.4). *Calliandra* plants selected for aluminium sleeves had no connectivity of branches to neighbouring plants in order to prevent access by terrestrial animals, as the smoothness of the aluminium prevents these animals from gripping and climbing towards flowers. All isolation experiments were conducted while *Calliandra* spp. flowers were in the budding stage, following Meyer (1989).

Figure 6.5. Aluminium sleeve encased around the base of a *Calliandra* plant with no canopy connectivity, to prevent access by non-flying mammals. Aluminium sleeves were supported with sticks tied together with gardening twine. Photo by KD Reinhardt.

6.2.4 STATISTICAL ANALYSES

As I collected all phenology measurements using ordinal values, I used LOESS (locally weighted scatterplot smoothing) to distinguish flowering trends of *Calliandra* plants across month of the year between 2015 and 2016. I used a Mann-Whitney U test to compare nectar availability (by proxy of flowering) of *C. calothrysus* and *C. tetragona* between the two years. I used a Friedman test to compare time devoted to nectar consumption by *N. javanicus* across the months of behavioural observations and a Chi-square test to compare the use of *C. calothrysus* and *C. tetragona* between activity budgets obtained for *N. javanicus*. All analyses were conducted using R 3.6.0; see table 2.7 in section 2.6 for program analyses packages.

6.3.1. NECTAR AVAILABILITY

Monitoring phenology of introduced food species available to slow lorises (see section 2.3.3), I monitored *C. calothrysus* (n = 146) and *C. tetragona* (n = 141) plants. In 2015, the dry months were from July until October, whereas in 2016 the dry months were from July until only September. *Calliandra tetragona* and *C. calothrysus* displayed significantly different annual phenology (Z= -4.19, p< 0.001). While *C. tetragona* displays a clear relationship between flowering phenology and seasons (dry versus wet; see section 2.2.2), *C. calothrysus* displays a more consistent annual presence of flowers through most of the year, as absence of flowers was only observed in September (the end of the dry period).

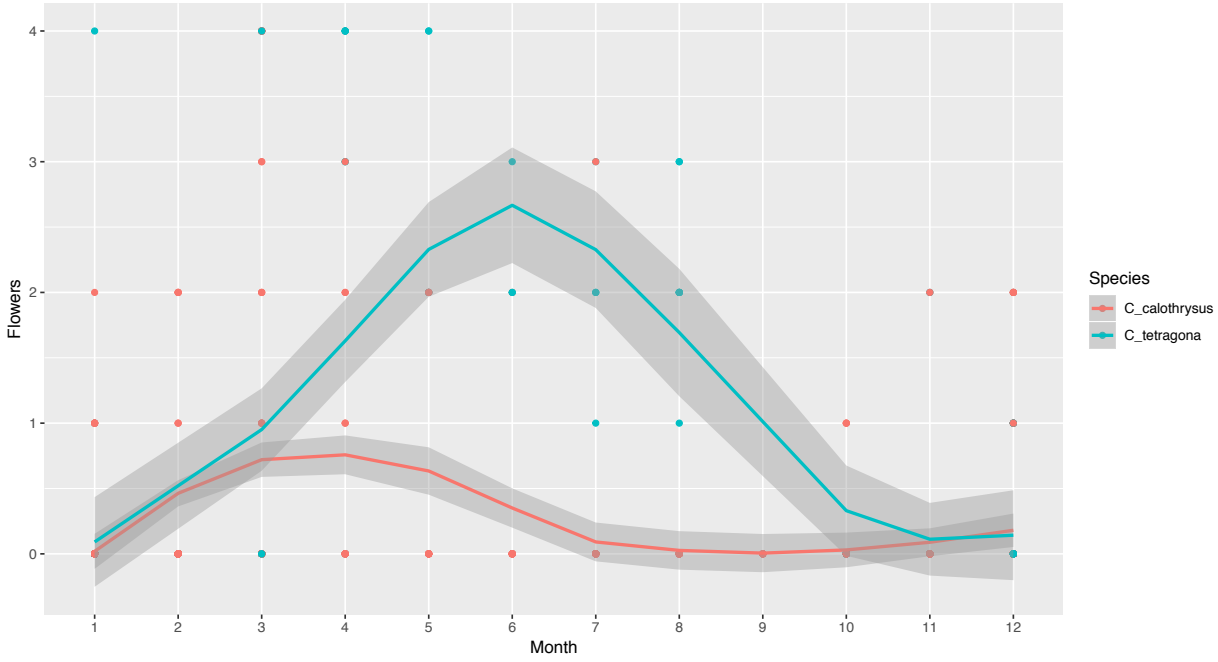


Figure 6.6. Flower phenology of all *Calliandra* plants monitored between July 2015 and October 2016, represented as a LOESS graph. The x-axis represents the months of the year, where 1=January and 12=December. The y-axis represents the phenology scale of flower production of plants (0—no visible flowers; 1—new buds, limited to a few branches (<50% of tree complements); 2—newly bloomed flowers (<50% of tree complements); 3—fully bloomed flowers (>50% of tree complements); 4—few bloomed/wilting flowers). Solid colour lines represent phenology trends, whereas the dark grey shading represent the smoothing effect of the lines and deviation from the norm. *Calliandra calothrysus* is represented in red and *C. tetragona* is represented in green.

6.3.2. JAVAN SLOW LORIS NECTAR FORAGING AND BEHAVIOUR

Between June 2015 and August 2018 when phenology was recorded, over 14,448 hours of behavioural data were collected. During this period, *N. javanicus* was observed to consume nectar (36% ± 6), insects (14% ± 6), gum (43% ± 15) and flowers (7%± 6) overall (derived from 5-minute instantaneous point samples; see section 2.2.2). A Friedman test showed that nectar consumption varied significantly across the months (X^2=36, df=1, p<0.001; figure 6.7). Data from 2018 was not included in statistical analyses, as it is an incomplete year of phenology data.

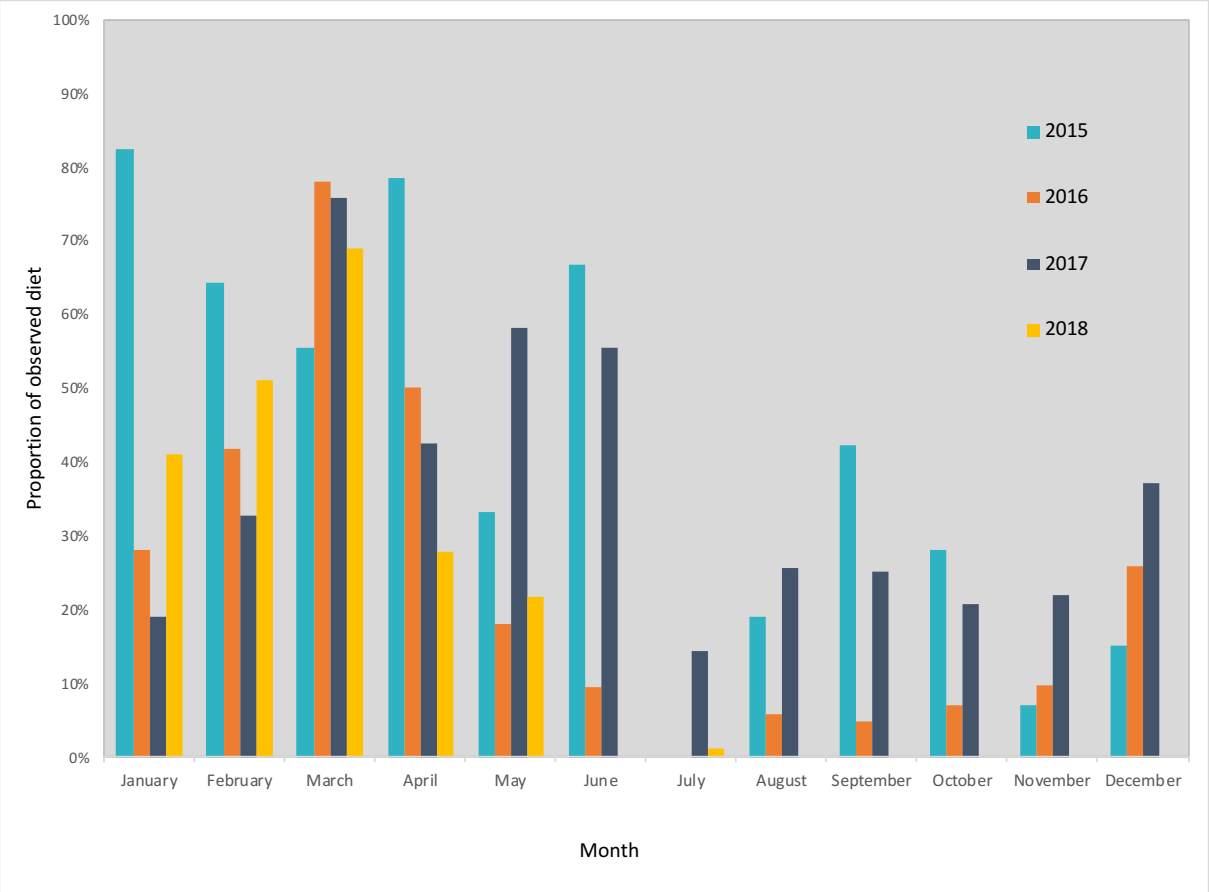


Figure 6.7. Proportion of *N. javanicus* diet observed to be nectar during behavioural observations of throughout the duration of this study.

Nycticebus javanicus was observed to use *C. calothrysus* significantly more than *C. tetragona* for feeding and foraging on nectar (X^2 = 3349, df= 10, p = 0.045), while spending 73.2% of total sample points spent feeding and foraging on *C. calothrysus* inflorescences (Figure 6.8). *Nycticebus javanicus* only used *C. tetragona* for <1% of time observed in all behavioural categories. Only one individual was every observed to feed on nectar from *C. tetragona*, and this individual was a juvenile male that only fed on nectar from a single inflorescence.

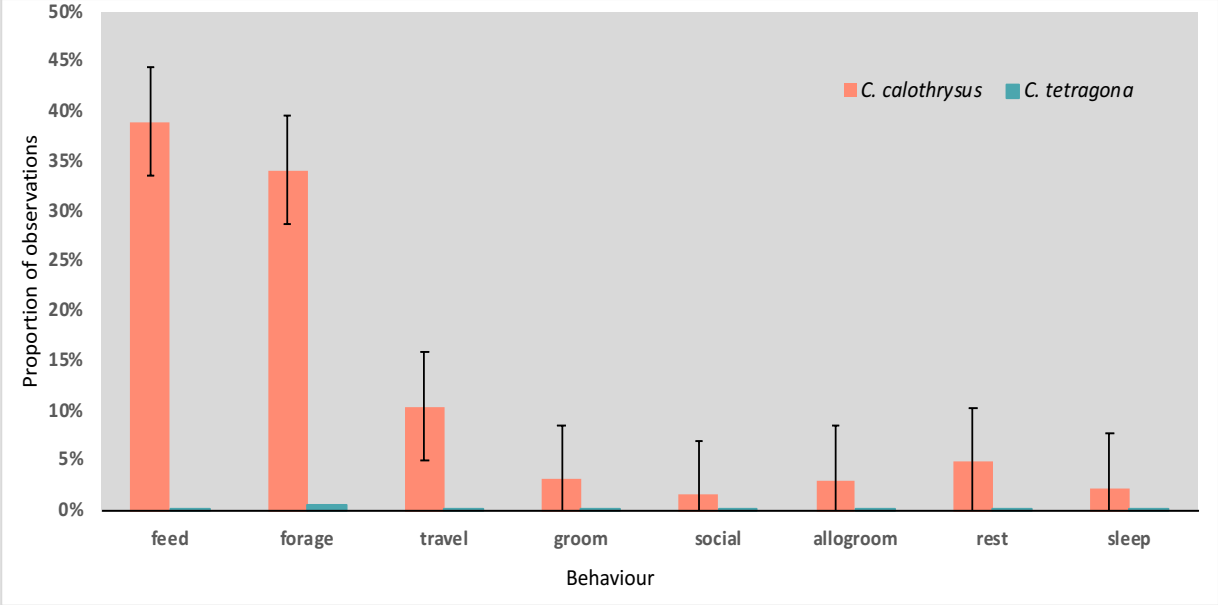


Figure 6.8. Activity budget profile of *N. javanicus* individuals (n = 34) using *Calliandra* plants over 14,448 hours of behavioural observations (mean values ± SE).

Nycticebus javanicus was observed to forage and feed on *C. calothrysus* using the mouth, uni-manual grasping, bi-manual grasping, reeling (see section 2.3.2) and various combinations of these techniques including the additional use of feet for grasping, to stabilise on terminal branches (Figure 6.9). Individuals predominantly foraged on *C. calothrysus* using a bi-manual grip (33.9%), a uni-manual grip (32.07%) and the mouth (19.29%). Time spent foraging on *C. calothrysus* ranged from 1 to 52 secs on a single

inflorescence, with an average of 139.8 ± 160 secs before leaving the plant, and locomoting to another.

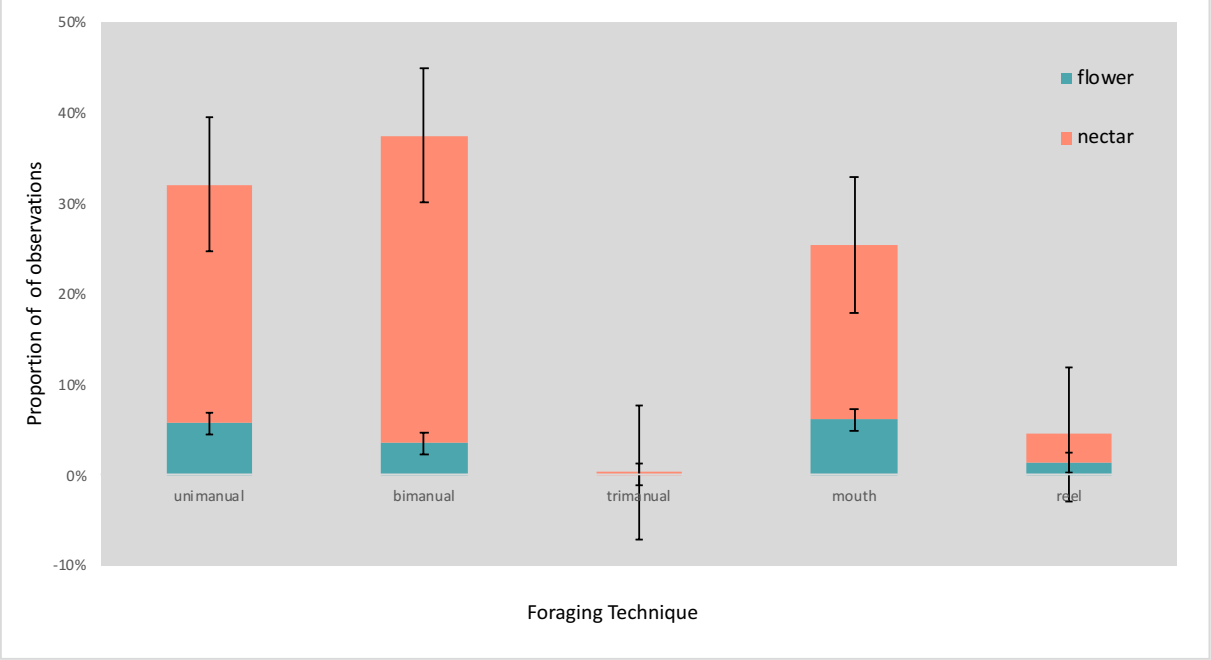
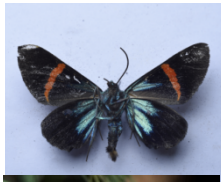
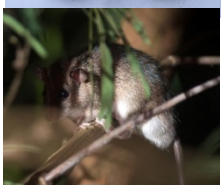


Figure 6.9. Foraging techniques of *N. javanicus* individuals (n=34) when foraging/feeding on *Calliandra calothrysus* during the overall study period (mean values ± SE).

6.3.3. POLLINATOR NETWORK

Over a total of 108 hours of all-night tree observations, native taxa observed to visit focal trees included moths (*Miliania* spp.), forest mice (pencil-tailed tree mouse (*Chiropodomys gliroides*), bats (Javan pipistrelle bat *Pipistrellus javanicus*, Horseshoe bat *Rhinolophus* spp.) and unidentified insect, where revisiting of individuals could not be distinguished (Table 6.3).

Table 6.3. List of all species observed to visit *C. calothrysus* plants during all-night tree observations (n = 9), between dusk and dawn in Cipaganti. I identified as close to the species level as possible, noting instances of observations, the duration of which individuals from each species visited the Calliandra plant (mean ± SD), if animals were detected by camera traps, and accompanying identification photographs. Photos by A. Walmsley, C.A. Henry, and W. Tarniwan.

Species	Visits observed (n)	Mean visit duration (seconds ± SD)	Identification Photograph
Milionia spp.	23	31.46 ± 25.6	
Chiropodomys gliroides	1	66	
Pipistrellus javanicus	1	152	N/A
Rhinolophus spp	16	9 ± 3.3	
Unidentified insect	1	20	N/A

While slow lorises were not observed to feed on *C. calothrysus* during the all-night tree observations, they were regularly observed to forage and feed on *C. calothrysus* nectar during nightly behavioural observations (see Figure 6.7 above). Examining pollinator exclusion plots, pollination bags (n=16) encasing inflorescences for both *Calliandra* species displayed significantly lower fruit-sets, suggesting that *Calliandra* is not a wind or self-pollinating genus. In comparison to open pollination plots, *C. calothrysus* plants covered with bird nets (*n*= 8) displayed a delayed phenology of 2-4 weeks with a reduced

fruit count in June, while *C. tetragona* covered with bird nets (n= 8) displayed no variation of phenology (Figure 6.9). Aluminium sleeves (n=6) used to exclude all non-flying mammals from *Calliandra* spp. displayed a shortened fruiting period in *C. calothrysus*, where flowers began wilting 1-2 months prior to other control plots. Aluminium sleeve control plots around *C. tetragona* (n= 4) had no visible effect on the phenology or fruit set of plants, although two had been cut by farmers.

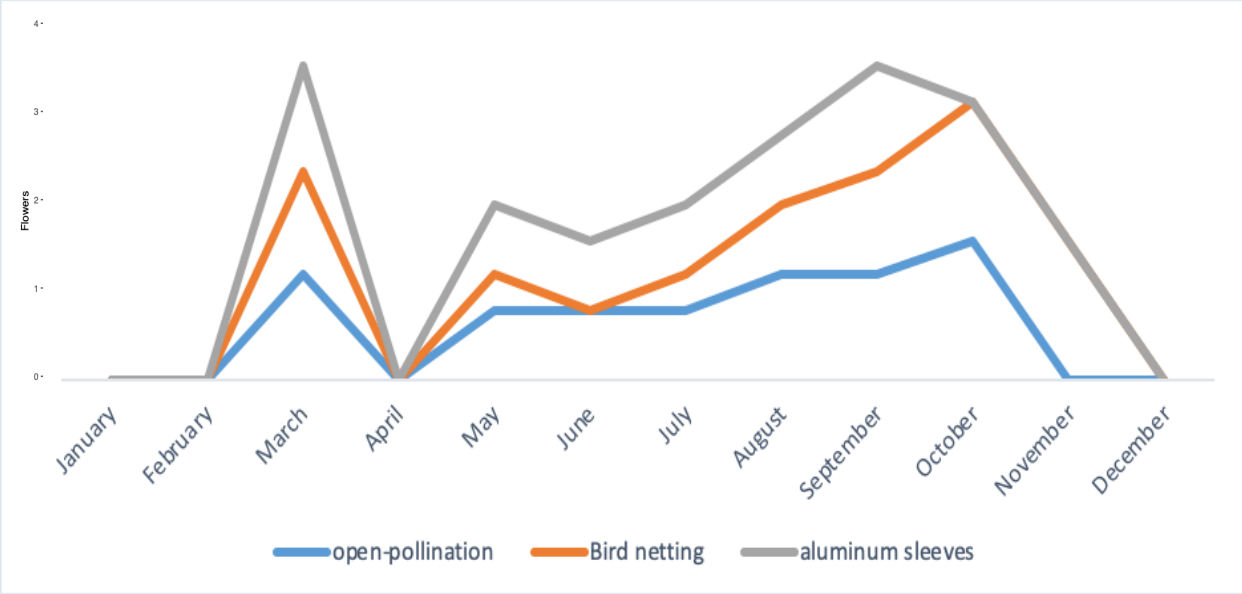


Figure 6.10. The flowering phenology of *Calliandra calothrysus* in the different control plots and open pollination plots, using the following phenology scale: 0 – no flowers; 1 – flower buds; 2 – new flowers; 3 – full bloom; 4 – wilting flowers.

During this study, introduced *C. calothrysus* was found to show low seasonality in flowering production, with a regular presence of flowers over 13 of the 16 months monitored. *Calliandra tetragona* was found to display a strong annual seasonality, where flowers were present for only half of the year. The constant presence of *C. calothrysus* flowers supports food access for *N. javanicus* during the majority of the year, when *N. javanicus* was observed to forage regularly on the nectar from this plant. Over the duration of this study, 36% ± 19 (annual average) of the *N. javanicus* diet was composed of *C. calothrysus* nectar, representing more than a third of the time attributed to energy intake. *Calliandra calothrysus* displayed a slightly larger pollinator network to other Calliandra species in endemic habitat, comprised of native endemic taxa (moths *Miliania* spp., Javan pipistrelle bat *Pipistrellus javanicus*, Horseshoe bat *Rhinolophus* spp., pencil-tailed tree mouse *Chiropodomys gliroides* and the Javan slow loris *N. javanicus*) that regularly revisit flowers. Pollinator-exclusion plots suggest each of these species contribute to the successful pollination and sustenance of *C. calothrysus* in this region, while non-flying mammals had the lowest impact on monthly flower phenology. The results of pollinator exclusion plots should be interpreted with discretion due to the low sample size of individual plots.

Calliandra calothrysus can produce flowers over many months of the year in particular climate regions, providing consistent food source that attract frequent and revisiting pollinators (Stephenson, 1981; Willson & Schemske, 1980). Over the duration of this study, Cipaganti experienced an average of 1-3 dry months per year, with no rainfall, similar to previous years (Reinhardt *et al.*, 2016). A dry period of 1-3 months is ideal for the flower productivity of *Calliandra* spp. in a given environment (Duguma and Tonye 1994). New

flower production was found to be continuous over an average of 10-11 months per year, making Cipaganti a most suitable habitat for the continuous success of this leguminous plant.

Nycticebus javanicus consumed nectar from *C. calothrysus* across 11.3 ± 0.6 months of the year, comprising more than one-third of their diet. This diet proportion is similar to that reported in previous studies of the same population (Cabana *et al.*, 2017) and for *N. javanicus* populations in Bogor, West Java (Moore, 2012) where slow lorises were observed to feed on *C. calothrysus*. Flying and non-flying mammals can play a beneficial and active role in the pollination of angiosperm plants whilst feeding on plant products (Carthew & Goldingay, 1997; Johnson *et al.*, 2001). In order for an animal to be a successful pollinator, they must transfer pollen between plants in the least destructive way. Researchers have found many species to contribute to dispersal of pollen to other plants as a result of pollen sticking to their fur or hair, and being transported to a different plant during regular behaviours (Lopezaraiza-Mikel *et al.*, 2007).

Aluminium pollinator-exclusion plots showed no influence on the phenology stages of *C. tetragona*, while *Calliandra calothrysus* experienced a delayed phenology when isolated from non-flying mammals. Observed foraging behaviour of *N. javanicus* to favour *C. calothrysus* therefore suggests that their feeding and foraging behaviour has a stronger contribution to the pollen network compared to its traveling behaviour. While many pollinators forage on various plant species, pollinators can distribute foreign pollen between species, which can clog the stigma and prevent germination (Waser, 1978). *Nycticebus javanicus* was never observed to forage or feed on *C. tetragona* plants, with the exception of one instance by a juvenile slow loris. While *N. javanicus* only used *C. tetragona*

for travelling, this does not disprove *N. javanicus* as a potential pollinator. However, exclusive feeding on *C. calothrysus* by *N. javanicus* would increase chances for successful germination, by decreasing the risk of transferring conspecific pollen loads between stigmas of *Calliandra*.

Nycticebus javanicus was observed to use multiple foraging techniques for consuming *C. calothrysus* nectar, including the use of their mouth, single-hand and double-handed grasping, and bimanual dexterity, where they reel a flower in towards their face. Visitation of pollinators to ecological substitutes can vary between native pollinators, particularly if introduced species display taxonomic affinity or shared evolutionary histories with native (or once native) flora, supporting morphology and foraging strategy of native pollinators (Menmott & Waser, 2002). *Calliandra* spp. flowers are located on the outermost tips of terminal branch inflorescences, undoubtedly requiring dexterity and a strong prehensile grip. The reeling method allows them to remain stable in terminal branches while lapping up nectar (Poindexter *et al.*, 2018) and releasing the inflorescence without harming the individual flower, supporting rewilding approaches between these taxa.

Calliandra produces more flowers than fruit, a strategy used to attract pollinators by increasing pollen relative to the number of ovules (Cruden, 1977). Researchers have found *C. calothrysus* fruit-set to be pollinator-limited in natural populations (Willson & Schemske, 1980). During this study, potential pollinators identified to contribute to the pollen network of *C. calothrysus* included moths (*Miliania* spp), bats (Javan pipistrelle bat *Pipistrellus javanicus*, Horseshoe bat (*Rhinolophus* spp.), forest mice (pencil-tailed tree mouse *Chiropodomys gliroides*) and the Javan slow loris (*N. javanicus*). This is a larger network than most *Calliandra* spp. in endemic habitats, with identified networks of bats,

birds and insects, but no record of non-flying mammals (e.g. *C. longipedicellata* in Mexico, Hernández-Conrique *et al.*, 2007).

While this study was conducted in a non-native environment, *Calliandra* spp. received various revisiting species and displayed changes in fruit-set in response to the prevention of access by some pollinator taxa, using pollinator-exclusion plots. If a plant is prevented access by specific visiting taxa and displays reduced reproductive success in response, this suggests that taxa play a significant pollinator role in the reproductive success of that plant, and that there is a competition for pollination between plants (Waser, 1983). Additionally, if the same mediated interaction results in an increased reproductive success, this suggests the plant facilitates pollinator visits (Rathcke, 1983). Although moths showed the highest visitor frequency during all-night tree observations, the results from the pollinator-exclusion plots suggested insects were not significant pollinators, as during this experiment the focal plays displayed no change in phenology. However, the bird netting used in this study had 15 x 15 mm squared holes, and it is possible moth wingspan exceeded these measurements, preventing these insects from accessing the control plots designed to test their influence as pollinators.

Researchers have found (generalist) native pollinators to readily pollinate non-native plants (Lopezaraiza-Mikel *et al.*, 2007; Richardson *et al.*, 2000; Waser *et al.*, 1996). This response can result in non-native plants being included in the pollination network of an ecosystem, supporting the fruiting and success of an invasive species (Lopezaraiza-Mikel *et al.* 2007, Stout *et al.*, 2006). Most studies examining plant-pollinator relationships of introduced species do not look at the community level, and the overall network between pollinators and multiple flowering plants in an ecosystem. Similarly, while this study recorded visitation

of native taxa visiting *C. calothrysus and C. tetragona*, it did not examine visitation frequency or duration of pollinator species to alternative flowering plants in the area (e.g. *Eucalyptus* spp.). Therefore, before *C. calothrysus* should be fully considered as a species for re-wilding, the relationship of pollinators with native and non-native species in a specific area should be considered.

One study conducted by Gérard *et al.* (2015) found introduced plant species as a method of rewilding to not only provide food and habitat for native animal species (including primates), but also contribute to local human livelihood. In particular, *C. calothrysus* has been planted in over 17,000 hectares of Indonesia as means of reforestation in villages where land is eroded with poor soil quality, or as preventative measures in steep areas where land is erosion-prone (Duguma & Tonye 1994). *Calliandra* species are adapted to moderately and highly acidic soils (Duguma and Tonye 1994). Contrary to most leguminous trees, *Calliandra* produces seeds best where it is introduced as an exotic species (Chamberlain & Rajaselvam, 1996). Indonesia has such successful *Calliandra* seed production that it is one of the main seed exporters to Sri Lanka and parts of Africa. Moreover, Indonesia is able to harvest and export more *Calliandra* seeds than countries where *Calliandra* is native, in South and Central America. This data supports the use of C. calothrysus as an ecological substitute for restoration or reforestation of habitat that is endemic to Javan slow lorises, particularly in area where similar plants that might have provided similar food resource have gone extinct or removed.

Despite non-native species being introduced to areas with intent of benefiting to a habitat, few introduced species have foregone cost-benefit analyses before being introduced, to know if an ecosystem will benefit from this introduction (McNeely, 2011). Should

introduced species have a negative invasive effect on a habitat, it can be extremely costly to eradicate these problems, or to compensate those affected, such as local farmers (McNeely, 2011). While *Calliandra calothrysus* has proven successful in West Java agricultural systems, introducing *C. calothrysus* into ecosystems inhabited by slow loris species elsewhere should be thoroughly investigated beforehand, including by performing cost-benefit analyses, habitat surveys and interviews with local residents/farmers.

6.5. CHAPTER SUMMARY

This chapter investigated the pollinator network of non-native leguminous plants (*Calliandra* spp.) in an agricultural ecosystem in Cipaganti, West Java, with a focus on the feeding and foraging behaviour of *N. javanicus* in nectar acquisition. Collectively, my results suggest that insects and flying and non-flying mammals are potential pollinators of *C. calothrysus* in this ecosystem. The observed potential pollinator community included moths (*Miliania* spp), bats (Javan pipistrelle bat *Pipistrellus javanicus*, Horseshoe bat *Rhinolophus* spp.), forest mice (pencil-tailed tree mouse *Chiropodomys gliroides*)and the Javan slow loris (*N. javanicus*). During behavioural observations, *N. javanicus* regularly engaged in non-destructive feeding bouts on *C. calothrysus*, further suggesting this primate contributes to the pollinator network. Furthermore, *C. calothrysus* isolated from non-flying mammals displayed a shortened flower and fruit periods. *Calliandra tetragona* showed no changed in phenology when isolated from non-flying mammals, suggesting *N. javanicus* and *C. gliroides* are not essential for successful fruiting in this species. Overall, *Calliandra* spp. displayed phenological shifts in response to being isolated from pollinators using exclusion plots, suggesting they are pollinator-dependent for successful reproduction. From these results, I suggest *C. calothrysus* be considered for conservation management practices in: a.) translocation of confiscated slow lorises into new habitat; b.) ecological substitute in re-wilding practices to restore ecosystems where slow lorises range; c.) *C. calothrysus* be encouraged as the species implemented in local agroforestry farming practices near slow loris home ranges.

CHAPTER 7 GENERAL CONCLUSIONS

In this chapter, I will discuss the various ways in which the data presented throughout this dissertation can both further our understanding of evolutionary pathways in the Order Primates, as well as support in conservation actions and policy for the Javan slow loris (*Nycticebus javanicus*). This research emphasises the importance of studying physiology in the wild, suggesting an intriguing possibility that some of our notions about physiological limitations and behavioural flexibility obtained in the lab or captive settings may not hold in natural habitats where environmental conditions are not stable.

7.1. CONSERVATION

The loss of species biodiversity is rapidly accelerating, calling for conservation action towards the protection and management of declining and endangered species and populations. *Nycticebus javanicus* is listed as Critically Endangered on the IUCN Red List (A2cd + 4cd) and has been included in the 'World's Top 25 Most Endangered Primates' since 2012 (Mittermeier *et al.*, 2012, 2014, 2016, 2018). Over the past 30 years, *N. javanicus* is suspected to have experienced a population decline of over 80%, resulting from illegal wildlife trade as profit for pets, tourist attractions (photo prop use) and traditional medicine (Li & Wang, 1999; Rode-Margono *et al.*, 2014; Nekaris & Jaffe, 2007). Due to the illegal trade of this species in conjunction with their level of endangerment, they are additionally classified within Appendix I of CITES (Conventional Trade on International Trade in Endangered Species of Wild Fauna and Flora) and Indonesian law (Nekaris & Nijman, 2007), which aim to diminish the level of trade of this species.

The majority of extinctions during the late Pleistocene were influenced by human intervention, where growing human populations in the current Anthropocene and unpredictable future land-use patterns pose a consistent threat to extinction rates (Donlan,

2005; Svenning *et al.*, 2016). As of 2009, a survey across the island of Java determined an estimated 17% of suitable habitat to remain within protected area (Thorn *et al.*, 2009), in addition to large populations of *N. javanicus* ranging in unprotected human-altered landscapes, such as agroforestry systems and villages (Voskamp *et al.*, 2014). As such, the success of conserving wild populations of *N. javanicus* is largely dependent on local communities and the adaptability of slow lorises to these mosaic landscapes and disturbed habitat.

Conservation science is a crisis discipline, where practitioners implement strategies and management policy to mitigate the loss of species, habitat and overall global biodiversity (Cooke & O'Connor, 2010; Soule, 1986). Increasingly, conservation science is embracing multidisciplinary research in order to manage and formulate feasible solutions and mitigation approaches towards conservation problems (Balmford & Cowling, 2006; Wilson *et al.*, 2015). In such, the disciplines of animal behaviour, physiology and conservation have increasingly received valid attention. With various wild taxa experiencing rapid decline in populations, evidence-based research is pertinent to understand the interactions between an animal's physiological/behavioural responses to environment conditions (Cooke & O'Connor, 2010; Sutherland *et al.*, 2004). And so, in 2006, Wikelski and Cooke formally coined the term "conservation physiology—the study of physiological responses of organisms to human alteration of the environment that might cause or contribute to population declines".

Conservation management often focuses on taxa at a population or species level, whereas physiology tends to examine responses on a smaller scale, from molecular to individual level. Often, evidence-based research in determining causes for a species' decline (as well as the consequences should decline continue) is ignored by policy makers, whom focus

more on experience. However, in 2007, Caro suggested that getting vital physiological information included in policy making requires concrete examples of how the research can inform conservation decision making.

In order for conservation physiology to be implemented by conservation policy makers, the parameters presented must be relevant, replicable (for comparative research) and minimally invasive (Cooke & O'Connor, 2010). Many species of taxa have proven difficult to observe and study in their natural environment throughout progressions of field biology, due to attributes such as difficult habitat terrain, cryptic behaviours or a broad range of geographic mobility (Kenward, 1987; Ropert-Coudert & Wilson, 2005; Serge, 2010). Progressions in biologging technology are allowing scientists to gain vital information on the ecophysiology of previously understudied species, in a minimally invasive manner (Cooke *et al.*, 2004; Langer & Fietz, 2014; Robert-Coudert & Wilson, 2005). Following suit of conservation physiology, throughout this dissertation I integrate the disciplines of primatology, physiology, neuroscience and ecology through (using biologging methods) in order to generate data that will be useful in conservation management and policy for *N. javanicus* populations.

7.1.1. THERMAL ECOLOGY

An individual's physiology is difficult to link to a population's overall fitness. In order for conservation practitioners to incorporate physiological information in management and decision making, physiological data must be applicable to populations and communities, either via proxy research or by understanding the parameters across multiple individuals or scale measurements (Cooke & O'Connor, 2010). In this dissertation, I measured the activity and sleep patterns, thermoregulation and assess the pollinator role of multiple *N.*

javanicus individuals in the same population and habitat. These individuals all displayed marked behavioural and physiological responses to their immediate environment, which should be considered in conservation policies, as described here.

One of the many aims of conservation physiology includes determining the abiotic factors that are consequential to the fitness of an organism, particularly resulting from anthropogenic influences (Franklin, 2009). In doing so, scientists can project physiological responses to forecasted environmental change. Trends in climate change suggest the overall atmospheric temperatures are increasing at drastic rates. Global mean temperatures are projected to increase between 1.7°C and 4°C by the year 2100, with temperature extremes increasing more rapidly (Meade *et al.*, 2018; Meehl *et al.*, 2007; Rahmstorf & Coumou, 2011). In **Chapter 3**, *N. javanicus* displayed lowering of T_{sk} (used as a proxy for T_b) to a degree that can be categorised as daily torpor expressions. Individuals in this population used torpor in response to colder ambient temperatures and limited resource availability as well as body mass. Individuals also displayed an upper T_{sk} limit, never exceeding 39°C in response to higher ambient temperatures. Should these climate projection rates hold, *N. javanicus* will need to respond using behavioural and/or physiological methods to cool their T_b, for maintaining homeostasis and reproductive success. Various Australian mammals tolerate increasing ambient temperatures and forest fires by storing heat via heterothermy to aid in evaporative cooling for consecutive hours (Meade *et al.*, 2018; Nowack *et al.*, 2016). There are limits, however, as to how long an individual can use this method before experiencing dehydration, varying across species. Furthermore, deforestation results in warmer climates, further restricting *N. javanicus* thermal ecological adjustments.

While *N. javanicus* seems to display adaptive flexibility in overlapping its home range with a human-altered agroforestry habitat, not all human-altered habitats maintain intercrops of trees that slow lorises are observed to use, as seen here. All species of Lorisinae are unique in that they perform non-saltatory arboreal climbing (Sellers, 1996). As a result, slow lorises must maintain some form of substrate contact at all times, restricting them in their locomotor routes and habitat preferences (Runestad, 1997). Such restrictions make them particularly vulnerable in fragmented habitats, demanding constant connectivity of substrates and routes in areas where there is presence of potential predators (Voskamp *et al.*, 2014). In **Chapter 4**, I presented data that suggests the selection of bamboo as sleeping sites by *N. javanicus* is due to its benefits in predator avoidance and microclimate buffer from the immediate atmosphere, serving as a form of thermal refuge. One of the main focuses of conservation physiology is to determine the ecophysiological constraints of organisms in response to anthropogenic change. This population of *N. javanicus* ranges in agricultural habitat, reflecting how other populations may also respond to such habitat changes.

Ecologists suggest microrefugia is difficult to both identify and protect (Ashcroft *et al.*, 2012; Morelli *et al.*, 2016). Despite these difficulties, the microclimate buffers that microrefuge provide are important for the physiological success of many species, supporting species abundance (Gillingham *et al.*, 2012a; Checa *et al.*, 2014), distribution (Martin, 2001; Kelly *et al.*, 2004), and behaviour (Cunningham *et al.*, 2015; Kelly *et al.*, 2004; Willis & Brigham, 2005). Researchers have found that manipulation of microclimate can be used to offset negative impacts on biodiversity in an ecosystem (Greenwood *et al.*, 2016), proving their importance for protection and focus in conservation (Bramer *et al.*, 2018). Therefore, the presence of bamboo in the home range of *N. javanicus* individual's

home ranges is crucial for maintaining physiological success in their natural environment, particularly in the current geological period of the Anthropocene, where wildlife is increasingly faced with modified habitats, from deforestation and agricultural expansion.

7.1.2. SLEEP

Specific characteristics of sleep reflect its numerous functions for the brain and overall physiology, overweighing the risks and disadvantages associated with time spent in sleep (Siegel, 2003). As discussed earlier, an important manifestation of sleep homeostasis is the capacity to compensate for the loss of sleep following its deprivation (Rechtschaffen, 1998; Tobler, 1985). Sleep deprivation may be caused by numerous stimuli, such as perceived predation, that cause arousal from sleep during regular circadian rhythms (Lima *et al.*, 2005). Similarly, stimuli can evoke arousal during physiological states of energy conservation, such as metabolic reduction (daily torpor or hibernation). Disturbance from regular sleep patterns can create a need for an animal to reallocate resting time at another portion of the day (i.e. sleep rebound). Sleep rebound suggests that sleep serves a restorative function, and that an animal can experience detrimental effects, should time for sleep be compromised (Rechtschaffen, 1998; Tobler, 1985; Rattenborg *et al.* 2004; Rechtschaffen & Bergmann, 2002; Siegel, 2003; Tonini & Cirelli, 2003).

Research on sleep deprivation and homeostatic balance has only been tested in laboratory animals which allow the control for numerous extrinsic factors affecting sleep, but do not consider the balance of sleep and energy conservation against the influences of fluctuating environmental factors in nature. Conservation Physiology requires regular comparative research between captive and wild organisms, to understand the full picture of an organism's physiological limitations and responses (Franklin, 2009). In **Chapter 5**, data

showed that *N. javanicus* displayed sleep patterns influenced by environmental factors (particularly, light and ambient temperature) as well as sleep homeostasis, suggesting that disruption from circadian sleep patterns would demand sleep rebound at another point in time. Fluctuations in ambient temperatures amidst sleep rebound (naps) disrupted the duration and depth of sleep, which could be detrimental for regulating sleep need. Without maintaining sleep need, an organism cannot maintain fitness, for survival. Sleep deprivation has been proven to result in loss of cognitive function (Kushida, 2004; McNamara *et al.*, 2010; Rechtschaffen, 1998). As ambient temperatures were found to influence both sleep patterns and naps, this suggests climate change trends could have detrimental effects on *N. javanicus*, similarly to their energy conservation and thermoregulation. Thus, comparative research in wild animals is pertinent to understand how animals balance both sleep and energy accumulation and allocation in nature.

7.1.3. ECOSYSTEM IMPACT

Conservation goals should not only seek to maintain healthy taxa populations within an environment, but also to understand and maintain any relevant role a population plays in the functioning and sustaining of an ecosystem (Soulé, 1986). In **Chapter 6**, I presented data on the role *N. javanicus* played in pollination of an intercrop species used by farmers to improve crop yields, *Calliandra calothrysus*. *Calliandra calothrysus* has proven to play an equally vital role in this ecosystem, providing food resources for many nectar foraging species. This mutual relationship between primates, plants and people in an agricultural landscape bring forward an interesting case for the conservation of pollinator species in an anthropogenically disturbed habitat with an introduced species. These relationships provide evidence relevant for both translocation of *N. javanicus* into new habitats, as well

as the introduction of *C. calothrysus* into habitats where *N. javanicus* ranges, as an ecological replacement.

Agroforestry practices meet demands of sustainable food production for the world's increasing human population while minimising land alterations or deforestation, decreasing overall adverse impacts on the environment (Akobundu, 1992). This makes agroforestry one of the main solutions towards sustainable agriculture, in maintaining food production demands without destroying natural resources according to the Food and Agriculture Organization of the United Nations (FAO) Technical Advisory Committee (TAC) (Treitz & Narain; 1988). In some cases, agroforestry systems have even been found to improve environmental quality through maintenance of genetic stability, decreasing pollution, reinforcing wildlife social systems, and replenishing ecological degradation (Harwood, 1987; TAC, 1989). The use of legume plant species in agricultural systems has been found to restore soil fertility and maintain organic matter, as well as prevent soil erosion and weed pressure (Akobundu, 1992). Specifically, legumes have been found to be most effective as perennial cover crops intercropped between crop rows, serving as Integrated Weed Management (IWM). IWM manages weed overtake without ecological or economic loss. As IWMs should also encourage crop growth without damage, IWM species should be field-tested in selected ecological areas.

Current conservation planning does not address the long-term potential rewilding has in restoration and evolution. Rewilding can restore the biodiversity of an area, which could have many potential outcomes, such as economic land value, tourism appeal, evolutionary and cultural benefits (Donlan, 2005; Lorimer *et al.*, 2015). That being said, this method does not come without potential for unexpected consequences. Rewilding can result in

disease transmission, similar to any translocation of a species into a new habitat, or negative impact on alternate native species where the rewilding is being practiced (Donlan, 2005). Therefore, similar to any reintroduction, rewilding should be assessed as to the positive and negative implications on a case-by-case and locality-by-locality basis.

A collaborative meeting was held between LFP and the local forestry department, Perhutani. The agricultural census covered 96 farms over two months where informal interviews were held with farmers to: i.) establish current farming practices; ii.) determine current crop and tree preferences and use; iii.) determine attitudes of farmers towards different species of flora; vi.) inquire about the willingness of farmers to participate in agroforestry programmes with LFP staff. A total of 37 farmers were interviewed by the LFP researchers during the agricultural census. All interviewed individuals unanimously expressed interest in receiving trees as part of an agroforestry programme. Farmers were open to working along with LFP staff to develop agroforestry strategies. Particularly, farmers and staff developed strategies in using *C. calothrysus* as a focal point, intercropping *Calliandra* plants with coffee. The aim of this strategy was to yield a higher profit for farmers in their coffee grade and crop yield. The second strategy for *Calliandra* use was to contour-plant individual flora between crops, to mitigate soil erosion and landslides. As local farmers expressed a strong interest in incorporating more *Calliandra* spp. in their regular agricultural practices, this suggests a mutualistic relationship between slow lorises and farmers in meeting agricultural demand (local livelihood) and conservation of a CR primate, as *C. calothrysus* in this region was proven to be a substantial component to the *N. javanicus* diet.

Nycticebus javanicus is regularly confiscated by CITES, intervening with the illegal wildlife trade (Nekaris & Jaffe, 2007). After confiscation, slow lorises are usually transported to nearby Asian rescue centres where individuals are assessed on their fitness for reintroduction back into the wild. Accelerating confiscations further result in rescue centres quickly reaching occupancy capacity for animals to be kept with ethical welfare standards, causing a domino effect for an acceleration of translocation efforts (Kleiman, 1989). Aside from species identification, the exact ecosystem from which individuals were initially captured is more than likely unknown, or undetermined. Therefore, animals must be translocated into new habitat, often resulting in low survival rates (Moore, 2014). Based on my results from **Chapter 6**, I propose *Calliandra calothrysus* as a method of naturalistic 'grazing' by *N. javanicus* and other species in west Javan agroforest landscapes. Furthermore, I propose the presence of *C. calothrysus* should be added to the IUCN Release Site Selection for translocations of *N. javanicus*, as an ecological measurement for locating suitable habitat. In conclusion, each of these chapters provide data as to how animals will physiologically respond to natural environments, which can be applied to evidence-based conservation management.

7.2 EVOLUTIONARY IMPLICATIONS

7.2.1. HETEROTHERMY

Expressions of torpor in the Order Primates were first described in 1981 by McCormick on *Cheirogaleus medius*, with majority of the proceeding research focused on Lemuridae—a group of strepsirrhines endemic to the island of Madagascar. Since then, researchers heavily focused on lemurs for physiological research, particularly Cheirogaleidae, which express seasonal hibernation (Blanco & Godfrey, 2014; Dausmann, 2008, 2014; Dausmann & Blanco, 2016; McCormick, 1981). In 2010, heterothermy was first verified in

a non-malagasy primate, with data displaying daily torpor use by *Galago moholi* (Nowack *et al.*, 2010). This was a major discovery for researchers interested in the evolutionary trends of heterothermy in primates, as focus was so heavily weighed on lemurs. One hypothesis proposed that heterothermy evolved prior to the divergence of Lemuridae, as an ancestral trait that likely aided the environmental stressors encountered in transit to colonise the island of Madagascar (60-50 mya; Kappeler, 2000; Martin, 1972). As Cheirogalidae is a basal group of Lemuriformes, this seemed the most probable hypothesis, with some species later evolving to use seasonal hibernation in the more seasonal habitats of Madagascar (Nowack & Dausmann, 2015). Considering the hypothesis of the colonisation of Madagascar, however, animals would be expected to experience extreme circumstances that would not allow the expression of torpor throughout the migration without regular disruption, causing arousal from the energy saving state (Masters *et al.*, 2007). Later studies claiming heterothermy to have evolved only 20 mya in Cheirogalidae suggest that heterothermy in primates evolved after lemurs had already colonised Madagascar, to cope with harsh environmental conditions across habitat types, as Cheirogalidae and *Microcebus* spp. display an incredibly broad range of torpor use and expression (Ohba *et al.*, 2016). After the discovery of torpor expression in *G. moholi*, however, the hypothesis for heterothermy to be an ancestral trait was strengthened.

While thermoregulation of Lorisidae have been investigated and described since 1975 (Müller), it was only in 2015 that the first physiological data were reported, with evidence of multi-day torpor use in a group of free-ranging captive *Nycticebus pygmaeus* (Ruf *et al.*, 2015). This was the first direct evidence of heterothermy in Lorisidae (Dausmann & Warnecke, 2017). This discovery strengthened the hypothesis that heterothermy in primates was an ancestral trait and less likely to be a representation of convergent

evolution, as Lorisidae diverged from Galagidae in the late Eocene/early Miocene (54.9—23 mya; Andrews *et al.*, 2016; Herrera & Davalos, 2016; López-Torres & Silcox, 2019; Munds *et al.*, 2018; Porter *et al.*, 1997; Pozzi *et al.*, 2014, 2015; Roos *et al.*, 2004; Seiffert *et al.*, 2018; Yoder, 1997; Yoder & Yang, 2004), which is before heterothermy is thought to have evolved in Cheirogalidae (Dausmann & Warnecke, 2017; Ohba *et al.*, 2016).

Data on torpor expression in *N. javanicus* not only supports hypotheses that heterothermy is an ancestral trait evolved to aid in coping with environmental stressors, but also supports hypotheses of lorisids originating from mainland Africa before the geological formation of Paratethys, which created a land bridge for migration of various species of mammals to Asia (Mein & Ginsburg, 1997; Chaimanee *et al.*, 2008; López-Torres & Silcox, 2019; Rögl, 1999; Sen *et al.*, 2013). Reasons for other species in mainland Africa and Asia not observed to express torpor could be due to lack of a need to use this physiological trait, as well as difficulty to study physiology in the wild. As mentioned earlier, conducting invasive physiology in the wild can be difficult, and especially complicated for endangered species, as many primate species currently are categorised (IUCN).

7.2.2. SLEEPING SITES

Phylogenetic analyses suggest that the use of bamboo (particularly as sleeping sites) emerged early in the evolutionary divergence of *Loris* and *Nycticebus*, where the use of bamboo as sleeping sites is absent in all Lorisiformes across both continental Africa and Asia, except for *Nycticebus* spp. (aside from *N. menagensis*) and *Galagoides demidovii* (Svensson *et al.*, 2018). This is likely due to the geographic distribution of bamboos across habitats, as *G. demidovii* is the only galagid observed to use bamboo as a sleeping site, and bamboo is present across the majority of its geographic range (Svensson *et al.*, 2018).

As bamboo was found to offer a thermal refuge from immediate ambient temperatures, it suggests they serve a similar function as tree holes or thermal refuge, used in heterothermic lemur species (Dausmann & Warnecke, 2016).

7.2.3. SLEEP

Studying sleep in the wild is essential to further our understanding of sleep ecology and evolution, filling an important gap in our knowledge of sleep across phylogeny in the wild. Curiosity towards the functions of sleep and its role in ensuring fitness of a species have driven the development of neuroscience research. From the evolutionary ecology viewpoint, sleep evolved not only to cope with immediate intrinsic homeostatic needs, but also as a response to predictable and unpredictable environmental conditions (Anderson, 1998; Siegel, 2005; Lima *et al.*, 2005). Therefore, it is natural to assume that the well-known phenotypic variability and flexibility in sleep patterns between and across species reflects the fact that it evolved in conjunction with species adapting to their habitat.

Mammalian studies have revealed that greater predation pressures have a negative effect on sleep, causing individuals to experience sleep loss or the need for polyphasic sleep patterns (Tobler, 1989; Lima *et al.*, 2005). By sleeping in frequent short bouts, an animal increases its level of consciousness and opportunities to monitor for predators (Capellini I., Nunn C.L., McNamara P., Preston B.T. and Barton R.A., 2008b; Voss, 2004). Ecology thus has a direct effect on the organization of sleep phasing patterns and sleep duration (the total time between when an individual goes to sleep and awakens) in mammals (Broughton, 1973, Lesku J.A., Roth T.C., Amlaner C.J. and Lima S.L., 2006; Lima *et al.*, 2005). Therefore, an animal's ecological niche and sleeping site location are hypothesized to have direct influence on the time constraint and limitations of both sleep duration and sleep cycle

(Capellini *et al.*, 2008b). Correlations between ecology and sleep duration remain virtually unknown, particularly in regard to aspects of predation risk and any impact it might have on adaptations in sleep cycles and cause for fluctuations (Lima *et al.*, 2005).

Mammals living in environments with high predation risk and exposure have been found to display less time sleeping (Zepelin, 2000), providing important implications for understanding predation effects on sleep architecture (Lima *et al.*, 2005). Correlations have also been found between predation risk and REM sleep, which is one of the deeper stages of sleep (Vyazovskiy and Delogu, 2014). REM allows an individual to spend less time sleeping by increasing sleep quality over less time (Siegel, 2005).

Most primate species display monophasic sleep patterns, with humans being the most representative (Lima *et al.*, 2005). Polyphasic sleep is hypothesized to be an ancestral trait of mammals that still characterizes smaller mammals with higher risks of predation (Capellini *et al.*, 2008b). The highly consolidated sleep of *N. javanicus* (Chapter 5) supports one of the five main characteristics of primate sleep, and is most likely due to strict nocturnal behaviour (Nunn *et al.*, 2010). The finding from Chapter 5 that slow lorises performed a relatively (to other nocturnal primates) shorter total sleep time, however, contradicts the assumption that diurnal primates have evolved to require less total sleep time, as a result of increased sleep intensity. Overall, sleep measurements of *N. javanicus* support hypotheses of predation pressure on sleep phasing. Furthermore, displaying a similar total sleep time to many haplorhines, *N. javanicus* ranging in Cipaganti are presumed to experience higher degrees of sleep intensity. Increased sleep intensity could be a result of low predation pressure, or the unique morphology of Lorisinae hands and feet (with a reduced second digit) combined with their retia mirabilia, or both. Thus,

displaying overall similarities in sleep patterns to haplorrhines and humans, these data supports hypotheses that primate sleep traits derived somewhere in the strepsirrhine clades.

Researchers have hypothesized that less disturbances during sleep increases the intensity of REM and NREM, known as the "sleep quality hypothesis" (McGrew, 2004; Samson, 2013). Comparative research on baboons and orangutans found that sleeping structures that promote specific sleep postures increase sleep quality (Samson and Shumaker, 2015). As mentioned before, slow lorises always sleep with their head tucked between their forelimbs and rolled in a ball (schlafkuegel or sleeping ball) (Ehrlich and Musicant, 1977; Glassman and Wells 1984; Rasmussen, 1986). This postural behaviour combined with their unique hand morphology and retia mirabilia allow slow lorises to cling onto a substrate while immobile for extended periods of time, without going numb (Ankel-Simons, 2000). These unique morphological traits aid to secure a grasp to swaying branches during strong winds, or defending against attacks and/or branch shaking from predators while demanding less energy exertion (Müller, 1985; Nekaris, 2014; Nekaris and Stevens, 2007; Starr and Nekaris, 2013). Such grasping abilities could form part of an anti-predator crypsis complex (Charles-Dominique, 1977; Oates, 1984; Nekaris, 2014). I suggest that the retia mirabile may have evolved to aid in antipredator strategies for clinging, while complimenting undisturbed sleep in slow lorises. Thus, the retia mirabile would serve a similar function to great ape nests or sleep platforms (Samson and Shumaker, 2015), further supporting the sleep quality hypothesis (McGrew, 2004; Samson, 2012).

7.2.4. NECTAR FEEDING AND MORPHOLOGY

Nectar feeding has commonly been observed to be an important food resource in slow loris diets, particularly for the Javan slow loris. This feeding process may be further aided by the sublingua (Figure 7.1), a second tongue (consisting of both muscle and cartilage) at the base of the primary tongue (Wood Jones, 1918). The sublingua is an anatomical feature present in all strepsirrhine primates and tarsiers (Cartmill, 2010; Hill, 1953). This structure is hypothesized to aid in cleaning the toothcomb—the fusion of incisors and canine teeth on the lower mandible (Ankel-Simons, 2007). It has also been hypothesised that primates coevolved with angiosperm plants, and that the sublingual may aid in lapping up nectar (Sussman & Raven, 1978; Sussman, 1991; Sussman *et al.*, 2013). This anatomical function would meet both taxonomic affinity between slow lorises and non-indigenous *Calliandra* flowers, displaying shared evolutionary histories of once native angiosperm plants (Menmott & Waser, 2002).

Figure 7.1 has been removed from this version of the thesis due to copyright restrictions

Figure 7.1. A Javan slow loris exposing its tongue. The outer square is a zoomed in photograph of the Sublingua—an anatomical feature all slow lorises, lemurs and galagos attain. Photograph taken at the Duke Lemur Center, NC, USA by David Haring.

7.3.1. LORISIFORMES

The use of accelerometers can help with wild research on nocturnal animals in general, especially for cryptic nocturnal primates, such as Asian and African lorises, galagos, tarsiers and lemurs. Asian and African lorises (lorisids), galagos, tarsiers and lemurs are cryptic primates with nocturnal activity patterns, making it difficult to maintain continuous and cohesive observations to measure and interpret behaviours and physiology. The use of accelerometers can provide a great deal of insight on the daily activity patterns of these primates in their natural environments.

Macrophysiology is the "investigation of variation in physiological traits over large geographical, temporal, and phylogenetic scales", and often referred to as a 'course-filter' approach (Chown & Gaston, 2008). By 1991, various researchers began validating external temperature-sensitive loggers in measuring skin temperature (T_{sk}) as an alternative method for measuring an animal's ecological physiology, tested on birds (Brigham, 1992; Thomas *et al.*, 1993) and bats (Austen & Thomas, 1996; Barclay *et al.*, 1996; Brown & Bernard, 1991). In 2005, Dausmann proposed that such bio-loggers could also provide a viable alternative in ecophysiology research on small non-flying mammals. Since then, the employment of T_{sk} measurements as a proxy for T_b has progressed, proving its reliability in measurements and providing great possibilities for advances in conservation research (Dausmann *et al.*, 2005; Nowak *et al.*, 2013; Wilson *et al.*, 2015). Investigating the ecophysiology of other lorisids and galagids via this approach would allow us to further assess not only a better understanding on the divergence of heterothermy and these primate taxa, but also provide insight as to how these groups will respond to future anthropogenic threats on a more robust scale, providing information beyond the organism

responses and instead focusing on a scale of population, epidemics and biodiversity problems. Thus, more studies on the ecophysiology of Lorisiformes would provide more applicable information for conservation practitioners and management planning at both a species and population levels.

7.3.2. HETERMOTHERMY AND SLEEP

As discussed earlier, sleep homeostasis is the compensation for lost sleep due to deprivation, which can be caused by numerous stimuli (Rechtschaffer, 1998; Tobler, 1985). Sleep rebound suggests that sleep serves a restorative function, and that an animal can experience detrimental effects, should time for sleep be compromised (Rechtschaffen, 1998; Tobler, 1985; Rattenborg *et al.* 2004; Rechtschaffen & Bergmann, 2002; Siegel, 2003; Tononi & Cirelli, 2003). Heterothermic species that display the use of daily torpor and hibernation have also been observed to perform sleep rebound post-arousal, suggesting an extra time pressure to balance energetics and the need to acquire sleep. Research on sleep deprivation and homeostatic balance has only been tested in laboratory animals which allow the control for numerous extrinsic factors affecting sleep, but do not consider the balance of sleep and energy conservation against the influences of fluctuating environmental factors in nature. As the data from this dissertation have proven *N. javanicus* to be a heterothermic primate that is capable of reallocating sleep when needed, it would be a good model species to test the balance of torpor expression and sleep rebound, across their circadian rhythms in the wild. This topic could not be investigated during this study due to numerous failed loggers, resulting in no individuals having both an accelerometer and a skin temperature logger on at the same time, but could be strategically investigated with synchronization of bio-loggers. Specific sleep stages (Proportions of REM and NREM sleep) should also be considered in future research, as they provide great insight into the quality of sleep, and physiological stages. This would be particularly interesting from a

thermal energy perspective. While endotherms can normally produce body heat to cope with minor weather changes, they lose thermoregulatory control during REM sleep (Manzar *et al.*, 2012). Therefore, it would be expected that slow lorises would need to balance their need to accumulate REM sleep with torpor expressions.

7.3.2. SLEEP QUALITY

The specialised grip of Lorisiformes aids in prolonged immobility during rest and sleep. These grasping traits may aid in prolonged grip during fur clinging in infants (Pouydebat, 2016). The ability to cling requires important grasping abilities and is essential to survive in species where the young are carried on the fur. It is hypothesised that fur-carrying species perform manual grasping of food items more frequently, as it is a precursor for manual dexterity (Bishop; Peckre *et al.*, 20106). Lorisines (*Loris* spp., *Nycticebus* spp.) are also fur-grippers as infants, which is correlated with their extended grip (Pouydebat 2016). This seems to have implications as well for the evolution of fur gripping, and correlates to sleeping site selection (Svensson *et al.*, 2018).

In conclusion, as slow lorises represent basal phylogeny (an early-branching clade where baseline expression of traits derived) in the Order Primates, they can provide great implications as to the evolutionary path of various physiological traits, from the divergence of heterothermy in primates, to the phylogenetic inertia of behavioural and physiological traits, such as activity and sleep patterns in humans. While the Javan slow loris is a seemingly specialized primate, they indeed express various traits that make them highly adaptable to anthropogenic disturbance, and to seasonal and climatic variation. The data presented in this thesis overall suggests that *N. javanicus* is highly adaptable to environmental changes, with a broad scale of thermal flexibility to colder temperatures, and the ability to adapt to new food resources in maintaining regular energy intake. In

conclusion, these data not only suggest that some of our basic notions about physiological responses obtained in the lab may vary in the wild, but can also be applied to rewilding conservation for this critically endangered primate.

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APPENDIX I. THESIS PUBLICATIONS AND COLLABORATIONS

Each of the below publications were submitted and published through peer-review during the time of this PhD, and were implemented with data and information collected throughout this dissertation.

LIST OF PUBLICATIONS

Chapter 1

Streicher, U. & Reinhardt, K.D. (2020) Thermoregulation in lorises. In: Evolution, Ecology and Conservation of Lorises and Pottos. Eds: Nekaris KAI & Burrows A. Cambridge/New York: Cambridge University Press.

Reinhardt, K.D. (2018) Hibernation. (Eds.) Vonk J & Shackelford TK, in: Encyclopedia of Animal Behavior and Cognition. Springer Publishing Co. DOI: 10.1007/978-3-319-47829-6_231-1

Reinhardt, K.D. & Nekaris, K.A.I. (2017) Long-Term Field Studies—Asia. (Eds.) Fuentes A, in: The International Encyclopedia of Primatology. Wiley Blackwell Publishing.

Chapter 2

Reinhardt, K.D., Campera, M. & Nekaris, K.A.I. (2020) Use of accelerometers in primate research. In: Evolution, Ecology and Conservation of Lorises and Pottos. Eds: Nekaris KAI & Burrows A. Cambridge/New York: Cambridge University Press.

Chapter 5

Reinhardt, K.D., Vyazovskiy, V.V., Hernandez-Aguilar, R.A., Imron, M.A. & Nekaris, K.A.I. (2019) Environment shapes activity and sleep patterns in a wild primate. Scientific Reports, 9: 9939. doi:10.1038/s41598-019-45852-2

LIST OF COLLABORATIONS

Suplemented by Chapters 4 and 5

Svensson, M.S. *et al.*, including Reinhardt, K.D. (2018) Sleep patterns, daytime predation and the evolution of diurnal sleep site selection in lorisiforms. American Journal of Physical Anthropology, 166(3): 563—577. DOI: 10.1002/ajpa.23450.

Supplemented by Chapter 6

Poindexter, S.P., Reinhardt, K.D., Nijman, V. & Nekaris, K.A.I. (2018) Slow lorises (*Nycticebus spp.*) display evidence of handedness in the wild and in captivity. Laterality: Asymmetries of Body, Brain and Cognition, 30: 1—7. DOI: 10.1080/1357650X.2018.1457046

Supplemented by Chapter 4

Nekaris, K.AI, Poindexter, S.P., Reinhardt, K.D., Sigaud, M., Cabana, F. & Nijman, V. (2017) Co-existence between primates and humans in a dynamic agroforestry landscape in West Java. International Journal of Primatology, 38(2): 303—320. DOI: 10.1007/s10764-017-9960-2

Several articles have been removed from this version of the thesis due to copyright restrictions. See Appendix I for a full list of publications

SCIENTIFIC REPORTS

OPEN

Environment shapes sleep patterns in a wild nocturnal primate

Kathleen D. Reinhardt[1,2], Vladyslav V. Vyazovskiy[3], R. Adriana Hernandez-Aguilar[2,4], Muhammad Ali Imron[5] & K. Anne-Isola Nekaris[1]

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Among primates, the suborder Haplorhini is considered to have evolved a consolidated monophasic sleep pattern, with diurnal species requiring a shorter sleep duration than nocturnal species. Only a few primate species have been systematically studied in their natural habitat where environmental variables, including temperature and light, have a major influence on sleep and activity patterns. Here we report the first sleep study on a nocturnal primate performed in the wild. We fitted seven wild Javan slow lorises (*Nycticebus javanicus*) in West Java, Indonesia with accelerometers that collected activity data, and installed climate loggers in each individual's home range to collect ambient temperature readings (over 321 days in total). All individuals showed a strictly nocturnal pattern of activity and displayed a striking synchronisation of onset and cessation of activity in relation to sunset and sunrise. The longest consolidated rest episodes were typically clustered near the beginning and towards the end of the light period, and this pattern was inversely related to daily fluctuations of the ambient temperature. The striking relationship between daily activity patterns, light levels and temperature suggests a major role of the environment in shaping the daily architecture of waking and sleep. We concluded that well-known phenotypic variability in daily sleep amount and architecture across species may represent an adaptation to changes in the environment. Our data suggest that the consolidated monophasic sleep patterns shaped by environmental pressures observed in slow lorises represent phylogenetic inertia in the evolution of sleep patterns in humans.

Sleep is a fundamental requirement for many animals in maintaining cognitive performance and physiological functions[1–3]. Birds and mammals can display two different sleep patterns: monophasic sleep, when an animal exhibits a single consolidated bout of sleep in one portion of a day; or polyphasic sleep, when an animal displays several short episodes of sleep[2,4,5]. Even in relatively stable laboratory conditions, the amount and characteristics of sleep and waking vary substantially across 24-h. These variations are superimposed with daily fluctuations in environmental variables that have a strong influence on activity patterns. Such fluctuations include risk of predation, ambient temperature, humidity and light[5–10]. The effect of these extrinsic factors on sleep and activity is mediated by their interaction with endogenous regulatory mechanisms, such as the circadian clock[11]. The circadian clock provides a rhythmic output to behaviour and physiology, and is synchronised to light levels, allowing animals to anticipate day and night[8,12]. This synchronisation is due to retinal photoreceptors and their photosensitivity to light cues, commonly referred to as Zeitgeber[8]. Another important intrinsic factor, which has a strong influence on sleep amount and intensity, is preceding sleep-wake architecture. Despite growing literature on sleep and its functions in the last decades, the need for comparative research in natural environments to further our understanding of sleep ecology, physiology and evolution is becoming increasingly important[2,5].

Numerous laboratory studies suggest that sleep is homeostatically regulated[13]. The intensity of sleep increases as a function of preceding wake duration, with lowest sleep pressure towards the end of a sleep period[14–17]. An important manifestation of sleep homeostasis is the capacity to compensate for the loss of sleep following sleep (or rest) deprivation[3,18]. An animal that experiences a stimulus that arouses them from sleep during regular circadian rest patterns would need to reallocate resting time at another portion of the day[5]. Sleep rebound is regularly observed in laboratory animals, and suggests importance of the restorative function of sleep, and the detriments an animal can experience should amount of time for sleep be compromised[3,18–22]. To our knowledge, no studies

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on instrumental sleep deprivation have been performed in the wild, where we surmise that the need to compensate for sleep loss would need to be balanced against environmental pressures[10]. One study conducted on wild African elephants (*Loxodonta africana*) found no evidence of sleep rebound in response to prolonged continuous spontaneous wakefulness[23]. Similarly, wild fur seals (*Callorhinus ursinus*) did not display REM sleep rebound when they returned to land after prolonged loss of REM sleep while in the water[24].

Sleep patterns in several mammalian species have been systematically studied in their natural habitat, but little is known about sleep in wild primates[23,25–29]. Evidence suggests that nocturnal primates display on average longer sleep durations (13–17 h daily) compared to diurnal species that sleep for 8–11 h[27,30]. Captive studies of nocturnal primates (African lesser bushbaby *Galago senegalensis*, greater slow loris *Nycticebus coucang*, Northern owl monkey *Aotus trivirgatus*) display strictly nocturnal activity[10], but it is unclear whether these patterns extend to the wild[31–34]. We aimed to bridge this knowledge gap on the environmental drivers of sleep in wild animals by conducting the first study on sleep patterns in a wild nocturnal primate, the Javan slow loris (*Nycticebus javanicus*).

We utilised actigraphy as a method to measure behavioural sleep (rest) of *N. javanicus* in the wild using collar-mounted accelerometer devices. Previous studies used activity monitoring in marmosets (*Callithrix jacchus*) to examine the diurnal rest-activity cycle[35] and this approach was validated against EEG recordings[36], confirming that actigraphy-defined immobility is a suitable proxy for sleep in primates. We hypothesised that under natural conditions, environmental variables would strongly influence the daily activity patterns and sleep behaviour of *N. javanicus*. We predicted that: 1.) *N. javanicus* would display diurnal sleep of a shorter duration than observed for nocturnal primates in captivity; 2.) *N. javanicus* would perform consolidated monophasic sleep patterns; 3.) Light and/or temperature would mediate their activity patterns; 4.) *N. javanicus* would display resting patterns that suggest homeostatic sleep regulation.

Materials and Methods

All research was conducted in adherence to the ethical practice and guidelines provided by the Association of the Study of Animal Behaviour, as well as the Indonesian Ministry of Science and Technology, RISTEK (1421/FRP/SM/VIII/2015). All research was additionally approved by the University Animal Ethics Sub-committee of Oxford Brookes University in the United Kingdom.

Study site and subjects. We studied *N. javanicus* on Mount Puntang on the Indonesian island of Java, which is part of the Java-Bali Rain Forests ecoregion. This population of slow lorises is found around Cipaganti in Garut Regency (7°16′44.30″S, 107°46′7.80″E, 1200 m asl), ranging between 1,250 and 2,364 m asl. This region is a sub montane environment near the equator, where temperatures vary greater on a daily range (typically between 16–35 °C) than they do annually[37].

The Javan slow lorises studied here are part of a population of wild individuals routinely monitored and studied *in situ* by an on-going (2012 – present) research project, the Little Fireface Project[38]. Individuals within this population have displayed a broad variation in home range size (2–19 ha[39]). They are routinely fitted with cable-tie radio transmitter VHF collars (Biotrack TW3, Wareham, United Kingdom) secured around their necks for individual identification. Due to the nature of slow loris locomotion (e.g. slow climbing, cantilevering), the placement of collars around the neck has been found most efficient for recording activity of *N. javanicus*[38,40]. To attach radio transmitter collars, animals are captured from trees by an experienced Indonesian field assistant using protective gloves. Capturing was done without anaesthesia and at a minimal frequency, to replace radio transmitter batteries (average 12-month battery life) or to retrieve accelerometers (average 3-month battery life).

All collared slow lorises were monitored on a regular rotating basis using Sika receivers and Yagi antennae (Biotrack Ltd., Wareham, United Kingdom) and head torches with red filters (Cluson Engineering Ltd., Hampshire, United Kingdom) between 17:00 and 05:00. Monitoring occurred an average of two nights per month using scan sampling to make sure collars remained properly fitted and individuals were performing natural behaviours[41].

Accelerometer recordings. To quantify activity and rest of *N. javanicus* we equipped electronic accelerometer devices (Actiwatch Mini: CamNtech Ltd., Cambridge, UK) to the VHF radio collars of twelve individuals on a rotating basis between June 2014 and April 2018 (see Fig. 1). Accelerometers were programmed to store full activity counts at 1-min epochs using MotionWare software (CamNtech Ltd., Cambridge, UK). Collar-mounted accelerometers have been found most suitable for wild primates because they do not disrupt their normal behaviours and are most convenient for long-term actigraphy monitoring[35,42]. The use of neck-collars is also best suited for comparative research conducted on arboreal primate species, as this method decreases the risk of animals getting caught in trees or branches[43–47]. The combined weight of radio-collars (19 g) and accelerometers remained well under the recommended five percent of the body weight of study animals, with *N. javanicus* body mass averaging at 903 g, and ranging between 850 and 1100 g[41,48].

Data processing. Raw data (activity scores) were extracted from accelerometer devices using the same software we used for launching the loggers (MotionWare software). Using the raw data, we analysed activity scores using Microsoft Excel and MATLAB version R2017b. While the methodology for activity recording in wild primates may need to be further improved in future studies, our data analysis suggests that the temporal resolution and sensitivity of our approach was optimal to capture animal's activity across a wide range of movement intensities and speed (see Figs 2 and 3).

Accelerometer devices record movement and locomotion as a complete activity score. One of the main defining characteristics of sleep is immobility, yet an animal may be awake while immobile[49–52]. While simultaneous behavioural observations can be used to extrapolate specific behaviours from activity scores, basic latent

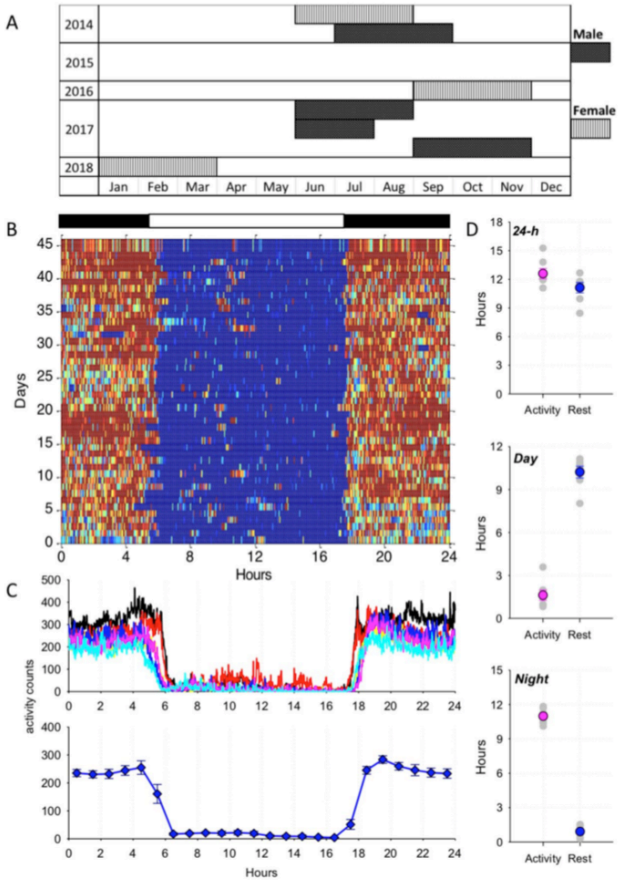

Figure 1. (**A**) Timeline of accelerometer recordings on individual animals (n = 7) shown separately for males and females. Each row represents one individual loris. (**B**) Representative 3-D actogram of the mean activity distribution of one individual loris across 24 h. Activity is represented in a colour gradient, where blue schematically depicts inactivity and red epochs correspond to activity. The bars above depict the night (hours between sunset and sunrise) and day (between sunrise and sunset). (**C**) Top: average activity profile plotted for each individual loris over 24-h; 1-min resolution. Each colour represents a different individual; bottom: average activity (SEM, n = 7) shown in 1-h intervals. (**D**) Proportion of time spent active and inactive (rest) over 24-h, during the day and during the night. Mean values (SEM) are shown as coloured symbols. Individual values are shown in light grey.

behaviours can be extrapolated from accelerometers using unsupervised algorithms for larger datasets or cryptic species[53–55].

We used actigraphy scores to devise criteria for behavioural sleep/rest[56–59], defining it as complete immobility (activity score equal to zero). Using survival curve analyses, we plotted the distribution of rest episodes during the night and during the day as a function of their progressively increasing duration, expressed as a percentage of the total number of episodes; we performed this same analysis for episodes of activity to quantify how long episodes with movement were sustained.

We refer to brief interruptions of rest lasting 5-min or less as "brief awakenings" (see Figs 4 and 5). Note that since no polysomnography data are available and the criteria are based on locomotor activity only, it cannot be determined whether the animals are awake during the entire duration of a 'brief awakening' epoch. To investigate the patterns of occurrence of brief awakenings during the day, we used data simulation approach of the retrieved complete loggers. To this end, we reshuffled brief awakenings from the same accelerometer recording, maintaining their quantity and duration but randomising their occurrence in simulated data.

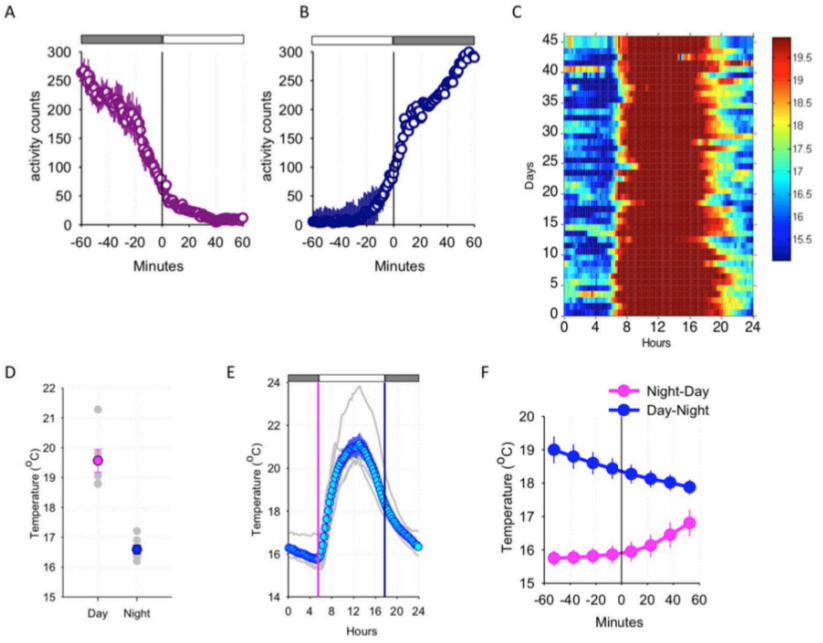

Figure 2. (**A**) The time course of locomotor activity at the transition from night to day (depicted as the bar above the curves). 1-min values of activity (first averaged between days within an individual, prior to calculating means between individual lorises, n = 7, SEM) are plotted for one hour prior to sunrise and one hour after the sunrise. (**B**) Same as A for the day-night transition. (**C**) Representative data of ambient temperature recorded from the home range of one individual loris across the entire recording period. The values are colour-coded according to temperature from warmer (dark red) to cooler (blue), as shown on the scale bar on the right. (**D**) Mean values of ambient temperature during the day and during the night. N = 6 animals, SEM. Individual values are shown in light grey. (**E**) The time course of average ambient temperature recorded from the home ranges of all individual lorises (n = 6, SEM). 15-min values are plotted consecutively from midnight till midnight next day. Grey curves represent ambient temperatures for each individual; blue circles represent the mean value for each 15-min temperature recording across a 24-h period. Vertical lines depict average time of sunrise (magenta) and sunset (dark blue). (**F**) Time course of ambient temperature during the corresponding 2-h intervals as shown in (**A**,**B**). Mean values (n = 6) are plotted in 15-min intervals.

Naps (periods of consolidated nocturnal inactivity) were defined as periods with zero activity lasting at least 10-min, which were not interrupted by transient epochs of activity longer than 2-min (see Fig. 6).

Our definition of naps equates to an arbitrary minimal duration of 10-min, and is based on extensive visual screening of the data. Generally, we observed an occurrence of consolidated periods of inactivity that were not interrupted by gross movements, if the animal was immobile for at least 10-min. Future studies may provide better criteria for naps when EEG recordings or other approaches to quantify sleep in slow lorises become available.

Measuring light environment and ambient temperatures. As light levels can be derived from sunrise and sunset, we gathered all sunrise and sunset times from an online world clock source (Time and Date AS 1995–2018) in the appropriate time zone (GMT +1) at the study site to test for circadian synchronisation. Ambient temperatures were extracted from Hygrochron iButton climate loggers (Maxim/Dallas Semiconductor Corp., USA) that were installed in each slow loris' home range. Loggers were placed out of direct sunlight on a lower tree branch for the full duration that individuals were fitted with accelerometers, and recorded measurements of ambient temperature at 5-min intervals with an accuracy of ±0.5 °C. Due to water exposure (rain and humidity) one of the retrieved climate loggers did not record data. The temperature measurements from one animal's home range therefore were not included in the analyses where temperature values were used.

Quantification and statistical analyses. Data analyses were performed using Microsoft Excel and MATLAB version R2017b. Group data are presented as mean ± SEM, or as individual points when showing representative data from a single animal. Each data representation type is specified in the respective legend for each figure.

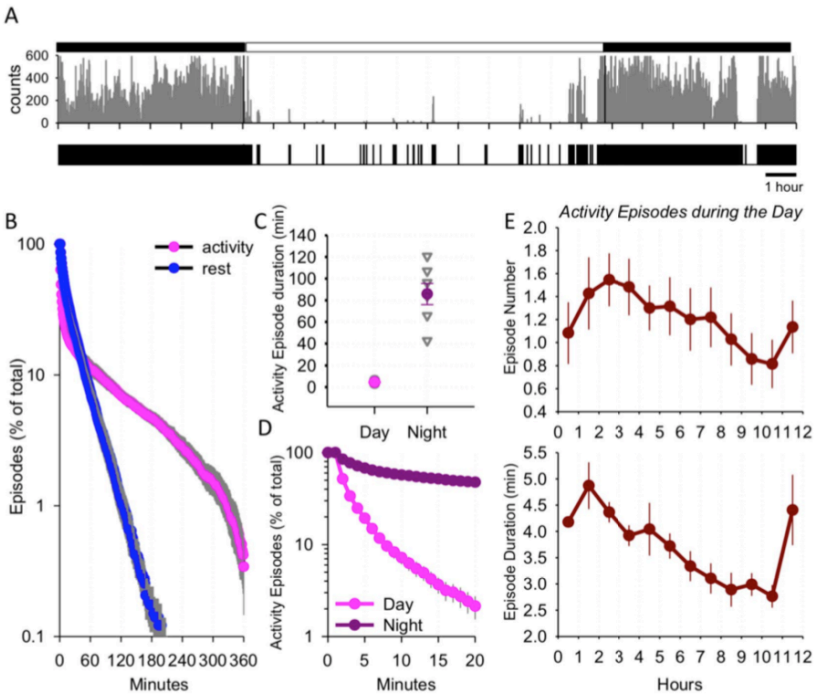

Figure 3. (**A**) Representative profile of activity shown across 24-h starting at midnight. The lower scale is a binary scale of the above measurements, where 0 is representative of complete immobility (no activity counts) and 1 corresponds to 1-min bins with at least one count of activity. Note that most activity occurs during the night, but occasional short activity bouts occur also between sunrise and sunset. (**B**) Distribution of rest episodes (defined as periods with 0 activity) as a function of their duration. Prior to calculating averages between days and individuals, all rest episodes were detected and plotted against their progressively increasing duration and expressed as % of the total number of rest episodes. Mean values, n = 7, SEM. (**C**) Mean duration of activity bouts during the day and night. SEM, n = 7. Individual values are shown as light grey symbols. (**D**) "Survival curves" of activity episodes during the day and night. The proportion of activity episodes is plotted as a function of their progressively increasing duration. Note that only a small fraction of activity episodes during the day "survives" beyond 5–10 min, while most activity episodes during the night are sustained for at least 20-min. Mean values, n = 7, SEM. (**E**) Top: time course of activity episode number during the day. The values are plotted in 1-h intervals from sunrise to sunset. Mean values, n = 7. Bottom: time course of activity episode duration during the day. The values are plotted in 1-h intervals as above.

We performed non-parametric Wilcoxon signed-rank tests to compare the mean proportion of time individuals spent in activity between the day and night (Fig. 1D), as well as the mean ambient temperature levels (Fig. 2D). We also compared ambient temperatures during bouts of activity/inactivity (Fig. 3C), and during the night with and without naps (Fig. 6E). We performed Repeated Measures ANOVA tests to assess changes in the duration of brief awakenings (Fig. 3E) and the duration of resting bouts (Fig. 5A) across time, between sunrise and sunset.

To test for homeostatic sleep regulation, we first measured the duration of sustained periods of locomotor inactivity as representative for rest consolidation during daytime rest, assuming that least disruptions from rest represent higher sleep intensity.

Results

Only seven of the twelve retrieved accelerometers had complete data stored. We omitted any loggers with saturated activity scores from our analyses, as these data were likely skewed by water damage, and non-skewed data could not be properly distinguished. Of the retrieved complete loggers, we acquired accelerometer data on four females and three males, of which six were adults and one was a juvenile (female). Retrieved accelerometers contained data for an average of 46.3 ± 0.8 days per logger, contributing to a collective 321 days of data between the individuals (Fig. 1A).

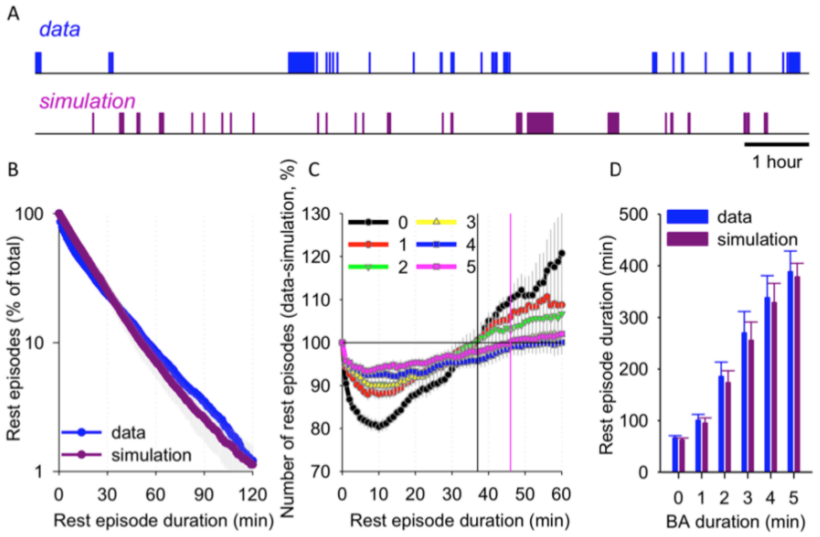

Figure 4. (**A**) Representative profile of activity (where 1-min bins with activity > 0 shown as 1) across one light period between sunrise and sunset. The plot below shows reshuffled activity periods of the same recording, where the number and duration of short activity bouts are retained as above, but their timing of occurrence is randomised. (**B**) Survival analysis of inactivity (rest) episodes for empirical data and simulated activity profiles as above. Note that shorter rest bouts are more likely to occur in the simulated activity time profiles, while longer sustained rest periods occur more frequently in the real data. (**C**) The difference in the distribution of rest episodes derived from data and simulation, calculated as in (**B**). An additional interruption criterion is introduced from 0–5 min. Vertical lines depict minimal rest episode duration where the rest episode duration in data is below simulation for rest episodes where no interruption is allowed (black) and where up to 5-min brief awakenings are permitted (magenta). (**D**) Mean duration of rest episodes derived from empirical and simulated data sets, shown as a function of the interruption criterion. Mean values, n = 7, SEM.

Light environment drives nocturnal activity and monophasic diurnal rest. Over the duration of this study, the average time (hh:mm ± SD) of sunrise was 05:49 ± 00:12 while sunset occurred at 17:51 ± 00:31. The amount of locomotor activity individuals performed was significantly lower during the day compared to night, where a striking consistency was observed between days with respect to activity offset and onset within and across individual lorises (Fig. 1B: representative individual; Fig. 1B,C: mean values). All animals displayed elevated continuous nocturnal activity pattern with prolonged periods of behavioural rest occurring exclusively during the day (Fig. 1C). The total daily amount of activity (defined as 1-min epochs with at least 1 activity count) and inactivity (defined as 1-min epochs with 0 activity counts) were on average similar (activity: 12.6 ± 0.5, rest: 11.1 ± 0.5 hours; $Z = 1.521$, $p = 0.128$), with periods of inactivity during the dark period being rare, and the reverse pattern displayed during the light period (Fig. 1D; $Z = 2.3664$, $p = 0.018$).

Changes in activity anticipated day-night transitions, where all animals typically displayed an onset and cessation of activity in close proximity to sunset and sunrise. Individuals began transitioning from active to inactive state approximately one hour prior to sunset (Fig. 2A) and transitioned from inactive to active state approximately 20-min prior to sunset (Fig. 2B), suggesting that the change in light levels were likely the key variable affecting the onset of activity and rest.

In addition to daily fluctuations in illumination levels, the ambient temperature also varied between the night and day, raising the possibility that it could also influence daily activity patterns, and trigger the onset of activity and rest. Our recordings revealed that during the day, ambient temperature displayed a mean value of 21.28 ± 0.92 °C, and a mean value of 16.90 ± 0.60 °C at night (Fig. 2D; $Z = 5.905$, $p < 0.001$). It was observed that ambient temperature values showed larger fluctuations during the day, peaking at around midday, while they were relatively stable during the night (Fig. 2C). The daily time course of temperature reached an average minimum around sunrise (15.33 ± 1.85 °C), while mean temperature reached an average maximum at midday (21.27 ± 0.39 °C; Fig. 2E). As ambient temperature transitions between the day and night were modest and typically did not exceed 1 or 2 °C (Fig. 2F), we surmise that transition of activity patterns are more likely driven by either anticipation of day and night or directly by changing levels of light.

Sleep can be quantified from immobile resting behaviour. Although prolonged bouts of inactivity are likely to represent sleep[60–63], the possibility remains that some portions of inactivity are merely quiet immobile wakefulness. To characterise the daily architecture of activity in lorises, first we used survival curve analyses

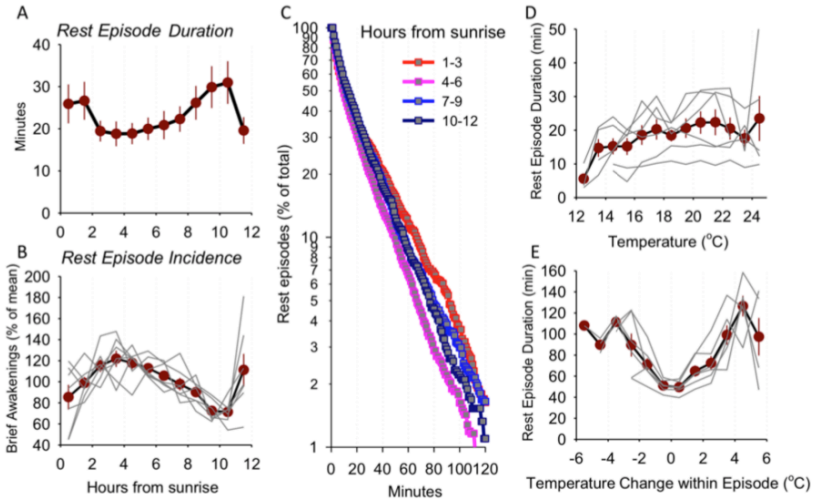

Figure 5. (**A**) Time course of rest episode duration across the light period. Mean values (n = 7, SEM) are shown as dark red symbols. (**B**) Time course of rest episode number across the light period. Mean values (n = 7, SEM) are shown as dark red symbols. The values are expressed as percentage of mean across the entire day. The curves for individual animals are shown in grey lines. (**C**) Survival analysis of inactivity (rest) episodes shown for 3-h intervals across the day. Note that rest episodes "survive" for longer at the beginning of the day, then their duration drops and tends to increase again in the second half of the light period. Mean values, SEM, n = 7. (**D**) The relationship between ambient temperature and rest episode duration during the day. All rest episodes during the day are grouped according to the ambient temperature at the time of their occurrence. Note that at warmer temperatures the rest episodes tend to be longer. (**E**) The relationship between change in ambient temperature within sustained rest episodes and their duration. All rest episodes are grouped according to the magnitude of change in temperature, and the corresponding values are averaged. Note that rest episodes tend to be longer if the temperature is decreasing or increasing, but remain short if the temperature is stable. Mean values, n = 7, SEM.

as an approach to assess continuity of activity and rest bouts (Fig. 3A). We observed that across 24-h, sustained periods of activity lasted significantly longer than sustained periods of rest ($Z = -2.366$, $p = 0.016$), and complete immobility episodes lasting for longer than approximately 2-h were extremely rare (Fig. 3B,D). This observation is consistent with previous studies in laboratory animals and humans, whose sleep is frequently punctuated with brief awakenings[56–59,64–68]. We next quantified the number and duration of brief awakenings occurring during the day, which revealed that these events occur on average 1–2 times per hour and mostly last <5 min (Fig. 3E; $F(11, 66) = 6.45$, $p < 0.001$).

While the occurrence of brief awakenings is an important feature of physiological sleep, they could also represent movement episodes occurring randomly during resting wakefulness[69]. To determine if daytime occurrences of activity represented brief awakenings from sleep, we tested the likelihood of animals to remain immobile longer than could be expected by chance. To this end, we shuffled the timing of all brief awakenings (with their corresponding durations) randomly across the day to quantify if the same distribution of rest bouts is observed in both the original and simulated datasets (Fig. 4A). The resulting time series suggested that randomly placed brief awakenings resulted in premature termination of prolonged rest bouts than is observed in the empirical data. To quantify these rest bouts, we plotted their distribution as a function of their duration for both the empirical and simulated data. We observed that the simulated dataset was more likely to contain short rest periods that lasted <30-min (Fig. 4B), indicating that rest bouts detected with actigraphy do not occur randomly between movement episodes, thus representing episodes of consolidated sleep. This effect was attenuated when interruption criteria were introduced (Fig. 4C). The lack of difference between the original and the simulated data sets suggests that the timing of brief arousal occurrence contains important information about rest consolidation, beyond merely rest episode duration (Fig. 4D).

Rest consolidation, intensity and naps are correlated with temperature changes. The key characteristic of physiological sleep is its homeostatic regulation, which is best represented by the levels of EEG slow-wave activity and the occurrence of consolidated periods of sleep or rest, less frequently interrupted by brief awakenings[69]. Calculating the occurrence of rest episode durations between the sunrise and sunset revealed that rest consolidation varied significantly over the course of the day, with longer rest episodes and lower incidences of brief awakenings at the beginning and towards the end of daytime rest (Fig. 5A–C; $F(11, 66) = 4.64$, $p = 0$). This

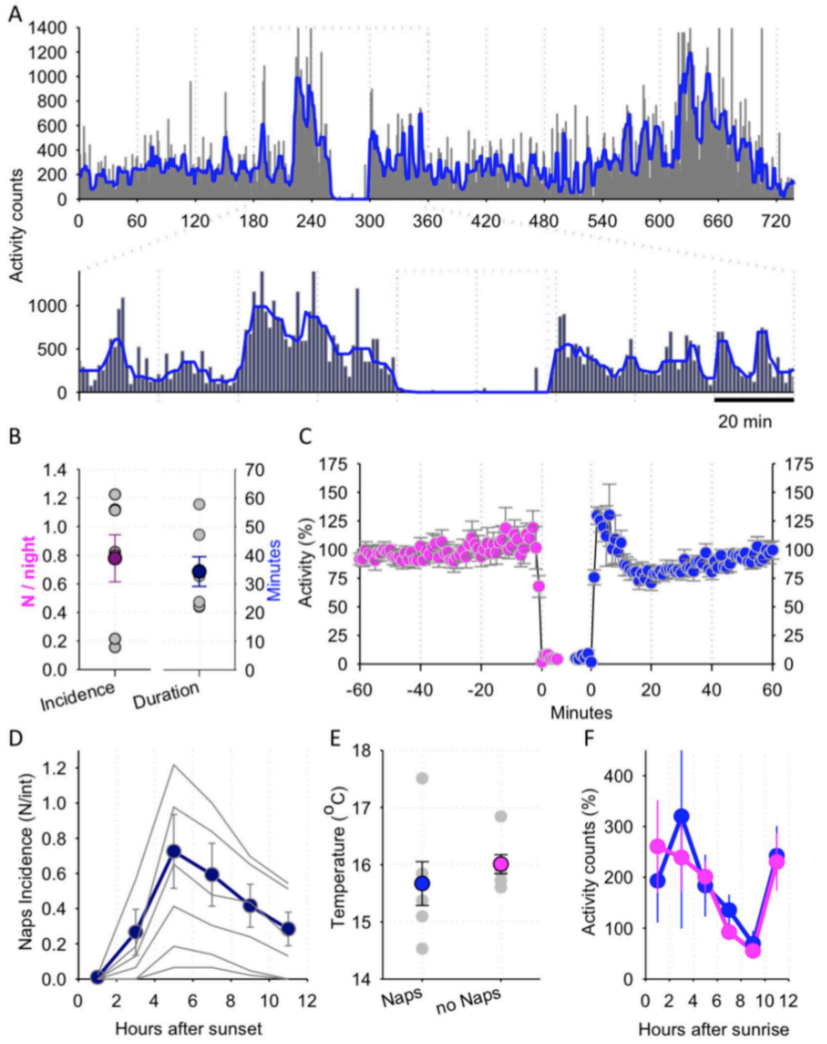

Figure 6. (**A**) Representative profile of activity shown across one night in one individual loris. Note the occurrence of a period of inactivity (nap) approximately 4.5-h after sunset (expanded below). (**B**) Mean values of nap incidence and duration during the night across the entire recording period. Mean values, SEM are shown as coloured symbols and individual animals (n = 7) are shown in grey. (**C**) The time course of activity around the nap. All naps lasting at least 10-min are aligned to their onset and offset and activity is averaged in 1-min bins over 1-h period before and after the nap. Note a surge of activity immediately after the nap. Mean values, n = 7, SEM. (**D**) The time course of nap incidence during the night. All naps lasting at least 10-min were detected across the entire recording period, and grouped according to the timing of their occurrence between sunset and sunrise. Note that the probability of nap occurrence is low during the early hours of the night, but increases progressively towards the middle of the night, and then decreases prior to morning hours. (**E**) The relationship between the occurrence of naps and ambient temperature. For each night across the entire recording period the occurrence of naps was determined and subsequently corresponding mean nightly temperature values were calculated separately for the night with at least one nap, and those nights where no naps occurred. Mean values, SEM are shown as coloured symbols and individual animals are shown in grey. (**F**) Time course of activity during the days following those nights where the animals took naps (blue curve) and those nights where the animals were continuously active. 2-h mean values (n = 7, SEM) of activity are represented as percentage of mean activity during the preceding day.

relationship further suggested that the architecture of rest periods, as detected by actigraphy, is not random, but varies consistently across the day. One possibility is that more consolidated rest reflects increased sleep pressure, which is expected to occur at the beginning of the habitual sleep period, as observed in other species, including primates[70,71]. However, we noted that this dynamic is also similar to the daily fluctuations in ambient temperature, which is at its lowest during the intervals when rest was most consolidated (Fig. 2E). To further assess the relationship between ambient temperature and rest episode characteristics, we clustered all rest episodes based on the average ambient temperature during their occurrence, which revealed a positive association. Specifically, rest duration was longer when ambient temperatures raised above 13 °C (Fig. 5D). Change in temperature within rest episodes also showed a relationship, with shortest episodes corresponding to stable temperature levels, but increasing along the steep upward and downward shifts in temperature (Fig. 5E). This result suggested that the duration of rest bouts may largely be determined by temperature levels and their instantaneous fluctuations, yet in some cases it may be partially uncoupled.

Finally, we noted that although the majority of night time activity periods were uninterrupted, the occurrence of consolidated periods of total or partial inactivity was not uncommon, and was encountered in all animals. While it is unknown whether such episodes of inactivity represent merely wakeful immobility or sleep, we tentatively referred to periods of immobility of $\geq$10 min as putative "naps". All animals displayed varying quantities and duration of naps in their regular activity patterns, ranging approximately between 10–60 min, with some individuals napping almost daily and others only occasionally (Fig. 6B). We observed a marked surge of activity after a nap (see individual example shown on Fig. 6A), which gradually decreased towards a lower plateau within approximately 20-min after a nap (Fig. 6C).

Next, we hypothesised that the occurrence of naps during the night may reflect increasing sleep pressure. To this end, we calculated the timing of nap occurrence. This analysis revealed that it was highly unlikely that an individual displayed a nap during the first few hours after sunset, while the probability of napping increased substantially towards the mid-portion of the night (Fig. 6D). The levels of ambient temperature did not show a strong association with the occurrence of naps (Fig. 6E; $Z = -1.363$, $p = 0.173$), suggesting that rather than being driven by changes in the environment it is likely to occur spontaneously. The occurrence of naps during the night was unrelated to the amount of activity during the preceding light phase (Fig. 6F), suggesting that their expression is dictated not only by preceding sleep-wake history, but possibly promoted when environmental conditions are favourable. To further investigate the influence of immediately preceding history of activity, we calculated the relationship between the intensity of movement 1-h prior to the first nap on each night and the corresponding duration of naps. In instances where more than one nap occurred during the night, we used the first nap only to avoid the effects of preceding naps, which sometimes occurred in "clusters". We found a weak positive association between the two (Pearson's correlation: $r = 0.29$, $p = 0.0008$), suggesting that nap characteristics may reflect sleep need. We emphasise the limitations when interpreting these results as further evidence for sleep homeostasis, as numerous environmental factors or internal factors may influence nap characteristics, such as body temperature, not accounted for with our approach.

Discussion

Our study is the first to describe and measure behavioural sleep of a nocturnal primate *Nycticebus javanicus* in their natural habitat. We observed that slow lorises displayed generally similar duration of immobility-defined sleep as diurnal primates, maintaining an average of eleven hours of sleep on a daily basis. The most important novel finding was that environmental variables, such as the levels of light and ambient temperature had a major influence, shaping the overall pattern of activity and rest across 24-h. Increased consolidation of rest at the beginning of their habitual sleep period may reflect increased sleep 'intensity' or direct influence of ambient temperature. Our study therefore raises an intriguing question of how wild animals cope with obtaining sufficient sleep, or compensate for sleep loss incurred during spontaneous or enforced wakefulness while adjusting their sleep pattern to predictable and unpredictable fluctuations in the environmental factors.

Environmental influences on circadian rhythm. Light environment can synchronise a mammal's circadian clock, regulating both behaviour and sleep[8]. Circadian rhythms allow an animal to predict regular changes in its environment, such as sunrise and sunset[72]. *Nycticebus javanicus* displayed circadian rhythms in activity and rest that were highly synchronised with the light levels. Anticipating sunrise and sunset suggests that in this species the circadian occurrence of activity and rest is strongly entrained to the periodicity of day and night.

In addition to the levels of light, the daily fluctuations of ambient temperature also played a role in sleep architecture. It has been reported in endotherms (including humans) that sleep and circadian rhythms can be altered when an individual is exposed to temperatures exceeding one's thermoneutral zone[5,25,73–76]. The combination of seasonal temperature shifts in conjunction with light level changes can result in dramatic shifts in activity patterns, as seen, for example, in the Arabian oryx (*Oryx leucoryx*)[25]. This species of oryx has been observed to prolong their time spent inactive during the winter when temperatures are cooler, in addition to showing an earlier offset of daily activity during the winter. We observed that slow lorises experienced elongated resting bouts during their daytime rest in response to warmer ambient temperatures. The strong effects of temperature on sleep in slow lorises have important implications for the conservation of this species, as remnant populations of *N. javanicus* are largely confined to high altitude habitats as a result of agricultural expansion and deforestation (less than 9% of forest remains on the island of Java). Temperature variation increases at higher altitudes[77], which would likely affect their sleep consolidation. Thus, if higher elevational gradients are the only remaining habitats for wild populations, slow lorises will likely experience lower sleep intensity.

Prolonged immobile behavioural rest and sleep intensity. Mammals and birds display homeostatic sleep regulation, where prolonged wakefulness is followed by prolonged rest during subsequent sleep[18,21,78]. Similarly, animals display sleep rebound where sleep is significantly disrupted mid-rest or when there is a loss

of sleep (sleep deprivation) altogether[17,20,79]. This rebound is acquired at a later period (such as during regular active periods), or extended on to the subsequent sleep. *Nycticebus javanicus* displayed prolonged immobility at the beginning of daytime behavioural rest, and this pattern suggests that animals experience a deeper sleep in the first portion of rest, following a period of activity, consistent with other studies in humans and laboratory animals[59,80]. Researchers have hypothesised that fewer disturbances during sleep increases the intensity of REM (Rapid Eye Movement) and NREM (non-REM) sleep, which is referred to as the "sleep quality hypothesis"[80–83]. Comparative research on baboons and orang-utans found that sleeping structures that promote specific sleep postures increase sleep quality[71]. A previous study on sleeping site selection on the population we studied found *N. javanicus* to select bamboo almost exclusively as sleep locations[39]. Lorises display unique vascularisation of their forelimbs (so called *rete mirabile*) that allow them to hold substrates for prolonged periods of time. Thus, in slow lorises the structure and size of bamboo as a sleeping substrate is in accordance with the sleep quality hypothesis, aiding undisturbed sleep[70,71,82]. We also observed that *N. javanicus* displayed a higher incidence of naps towards the middle of the night—their usual active period. As there was no indication that nap occurrence was triggered by changes in ambient temperature or light levels, it is possible that these naps reflect increased sleep propensity, indicative of a higher sleep need accumulated from continuous activity. The occurrence of these periods of inactivity during the night was unrelated to the total amount of activity during the preceding day, suggesting that extrinsic factors are an important determinant of napping, above and beyond the intrinsic sleep need. This adaptation also suggests a trade-off of foraging and social behaviour for sleep.

Accelerometers can provide non-invasive insight on sleep patterns of wild animals. While polysomnography (the recording of EEG, EMG and EOG) is considered the most accurate and efficient method for sleep measurements, it can be particularly difficult to conduct in the wild, especially with primates, leaving gaps in our knowledge on the accurate details of primate sleep in their natural environments[30,71]. The majority of sleep quotas and sleep quality data have been collected from captivity, with a paucity of information from mammals in their natural habitats. Kavanau and Peters[11] found African and Asian lorisids to display strict nocturnal patterns in captivity, beginning cessation of activity an average of thirty minutes prior to artificial sunrise. We found *N. javanicus* to begin cessation of activity approximately one hour prior to sunrise in the wild, displaying earlier anticipation of their light environment than in captivity, suggesting a greater sensitivity to natural light. Therefore, actigraphy represents a very useful tool for ecological research, especially considering highly threatened species for which invasive methods would not be permitted or ethical[84,85]. While we regularly refer to resting bouts as a proxy for sleep, measurements of sleep were derived from accelerometers only, and future studies are necessary to validate immobility in defining sleep in this species. Keeping this limitation in mind, our study suggests that important insights about daily architecture and regulation of wake and sleep can be obtained non-invasively.

Sleep patterns in wild primates. *Nycticebus javanicus* displayed highly consolidated monophasic sleep patterns. Sleeping at distinct times of day (in this case, dawn until dusk), *N. javanicus* conformed to typical patterns of a nocturnal, monophasic mammal[4,5,86]. Most non-human primate species display monophasic sleep patterns with polyphasic sleep patterns typically exhibited by small-bodied rodents and insectivorous mammals[5]. These tendencies are suggested to be both largely due to the metabolic processes unique to sleep[73], as well as tendencies to be prey to many predators where periodic wakefulness can increase predator avoidance[31]. Researchers have reconstructed polyphasic sleep patterns to be the ancestral trait in mammals[87], and have suggested that monophasic sleep is a derived trait in the suborder Haplorhini[30]. Our study performed in a nocturnal primate belonging to the basal primate clade, Lorisiformes, suggested that pressures for monophasic sleep also occurred in slow lorises. These results show sleep patterns that likely characterised the earliest primates, challenging the assumption that monophasic sleep arose in the diurnal primates and suggesting that human sleep patterns have a longer evolutionary history than previously suggested. Thus, our findings offer a reassessment of the evolution of sleep architecture in primates.

For monophasic sleepers, the nocturnal species studied so far display longer sleep duration than diurnal species. These studies, however, were conducted in captive settings, and so do not fully reflect sleep behaviour occurring in the environment where predation, food resources, climatic shifts and changing levels of sunlight are considerable restrictions on the amount of time an animal can spend in sleep. Monophasic sleep is suggested to be more efficient because it involves more time in deep sleep, thus requiring a lower total sleep time per day to meet sleep requirements [31; 88]. Our finding that slow lorises perform a relatively shorter total sleep duration in the wild (~11 h daily) compared to other nocturnal primates (ranging from 13–17 h daily in captivity) contradicts the assumption that diurnal primates have evolved to require less total sleep time, as a result of increased sleep intensity[27,30,88]. Overall, *N. javanicus* displayed activity patterns that are strikingly synchronised with sunset and sunrise, and rest fragmentation and duration that are correlated to temperature changes. Our results urge more research on the sleep patterns of other wild mammals, particularly primates, in testing hypotheses on sleep traits and how they may be influenced by changes in the natural environment.

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Author Contributions

K.D.R. designed and performed the study, data analysis and wrote the paper; V.V.V. designed figures and performed theoretical analyses, and contributed to manuscript preparation; R.A.H.A. and M.A.I. advised on experimental design and contributed to manuscript preparation; K.A.I.N. supervised the project, secured the funding for and runs the long-term study site. All authors read and approved the manuscript for submission.

Additional Information

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